THE EFFECTS OF PREDNISONE ON PROTEINURIA

Methodological studies and therapeutic aspects in patients with membranous glomerulonephritis

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E bóch kan nit zoeë sjleët zieë
of me weëd jet loeëzer derva

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CONTENTS

CHAPTER I

GENERAL INTRODUCTION.

From 1980 we have treated patients with idiopathic membranous glomerulonephritis with high-dose alternate-day prednisone according to the protocol of the Collaborative Study of the Adult Idiopathic Nephrotic Syndrome [1]. While treating these patients we observed a typical fluctuating pattern of proteinuria, due to an increased protein excretion on prednisone days and a decreased proteinuria on non-prednisone days [2]. To explain this peculiar phenomenon we have systematically studied the acute effects of corticosteroid treatment on proteinuria in patients with idiopathic membranous glomerulonephritis. These studies, described in chapters VI-VIII, form the main part of this thesis. In chapter II we review facts on idiopathic membranous glomerulonephritis, its natural history and treatment modalities. Factors related to the measurements of proteinuria and renal function in patients with renal diseases are described in chapters III-V. Finally, we address the course of the disease and possible benefits of treatment with corticosteroids and other immunosuppressive drugs in our patients in chapters IX and X.

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CHAPTER II

MEMBRANOUS GLOMERULONEPHRITIS: A REVIEW

Membranous glomerulonephritis is a chronic glomerular disease with a well-defined histopathological picture, characterized by the presence of subepithelial immune deposits on electron microscopy [1]. Under light microscopy the glomeruli show thickening of the capillary wall in the absence of cellular proliferation. Silver stains show densely staining projections into the basement membrane known as "spikes". Presumably these spikes represent normal basement membrane extending between the abovementioned deposits. In immunofluorescence microscopy granular deposits containing IgG and C3 are found in the capillary walls.

Although the incidence of membranous glomerulonephritis is rather low [approximately 10 new cases/million/year] [2], it is the most common cause of the nephrotic syndrome in adults, being the underlying glomerular disorder in about a quarter of nephrotic patients [1]. Membranous glomerulonephritis may be associated with a number of underlying diseases or toxins [table I], but in most cases [70 - 85%] no underlying cause can

Table I. Causes of secondary membranous glomerulonephritis

Malignancies:	carcinoma (lung, breast, colon) lymphoma
Infections:	hepatitis B syphilis malaria filariasis
Drugs:	gold penicillamine captopril NSAID
Auto-immune diseases:	SLE thyreoiditis
Miscellaneous:	sarcoidosis diabetes mellitus

Adapted from ref. 1, 14, and 48

be identified [idiopathic membranous glomerulonephritis]. Familial occurrence of idiopathic membranous glomerulonephritis has been described in rare cases [1,3]. In Caucasians idiopathic membranous glomerulonephritis has been associated with the histocompatibility antigens DR3, B18, and B8 [4-6], whereas from Japan a predominant association with DR2 was reported [7].

NATURAL HISTORY OF IDIOPATHIC MEMBRANOUS GLOMERULONEPHRITIS.

Idiopathic membranous glomerulonephritis occurs most commonly in adults between 30 and 50 years of age [8]. Males are more frequently affected than females in a ratio of 2:1. The short-term outcome [<five years] of membranous glomerulonephritis has been extensively reported on [9-16]. However, it is difficult to compare the results because most of the older studies suffer from the handicap that not only patients with secondary forms of membranous glomerulonephritis were included, but also treated as well as untreated patients. Furthermore the studies are quite heterogeneous regarding the proportions of female patients, the number of patients presenting with asymptomatic proteinuria, and the prevalence of other prognostic factors. In the abovementioned studies complete remission of proteinuria occurs in about 16% to 29% of patients, whereas about 40-60% of patients show evidence of progressive renal insufficiency, up to 38% reaching end stage renal disease [ESRD] within five years after onset of the disease. In table II an overview of recent studies on the outcome of idiopathic membranous glomerulonephritis in untreated patients is presented. It is evident that the results of these studies are quite variable, the percentage of patients entering a complete or partial remission of proteinuria ranging from about 20% to 65%. Likewise the percentage of patients, developing renal insufficiency varies from nearly 20% to over 50%. Also, the rate of progression is quite variable. However, overall the disease follows a rather indolent course, only 5-23% of patients reaching ESRD within five years after presentation. From these studies it is evident that time of follow-up is rather important, the frequency of

Table II. Outcome of idiopathic membranous glomerulonephritis in untreated patients

Pat. (No.)	Sex (M/F)	Age (years)	NS (%)	Screat at presentation (μmol/l)	Screat at presentation (% abn.)	Follow-up (years)	Outcome: CR (%)	Outcome: PR (%)	Outcome: RFD (%)	Outcome: Doubling of Screat after two years (%)	Outcome: ESRD within five years (%)	Ref.
116	60/56	38 (5-80)	76	ND	6	4.5 (0.2-21.5)	23.5	14.5	19	12	6	17
64	47/17	45 (20-70)	81	110	33	7 (2-15)	ND	ND	52	12.5	23	18
38	20/18	35 (16-65)	100	90 (±18)		2 (0.3-4.3)	10.5	7.9	>29	36	15.8	19
77	44/33	45 (16-83)	73	103 (±9)	23	4.0 (2-10)	35	30	25	<10	5.2	20
39	29/10	42 (16-74)	100	93 (±25)		5.0 (2-11)	5	18	>49	13	8-12	21
89	56/33	50 (11-81)	83	116 (±50)		6.1 (0.2-14)	——57——		33	12	16	22
37	31/6	42 (17-72)	93	130 (50-660)	16	5.3 (2.5-9.3)	22	8	47	ND	ND	23

Abbreviations: M=male; F=female; NS=nephrotic syndrome; Screat=serum creatinine; abn=abnormal; CR=complete remission of proteinuria; PR=partial remission; RFD=renal function deterioration. ND=no data

Table III. Long-term outcome in idiopathic membranous glomerulonephritis

Patients (No.)	Sex (M/F)	Age (years)	NS (%)	SCreat at presentation (μmol/l)	SCreat at presentation (% abnormal)	Follow up (years)	Outcome CR (%)	Outcome PR (%)	Outcome RFD (%)	Ref.
35	18/17	36 (7-69)	74	ND	45.7	14 (10-20)	29	23	43	24
49	37/12	47 (13-70)	100	108±27		9.5 (2-26)	14.3	40	45	25
104	64/40	40 (15-81)	68.3	ND	20	11.5 (1.0-24)	40	30	30	26

Abbreviations: see table II.

patients going into complete remission increasing with time. In Cattran's study the percentage of patients with a complete remission increased from 15% at one year to 35% after four years [20]. In the study of Noel a complete remission was reached after on average 5.1 years of follow-up [17], whereas Ponticelli did not observe a single complete remission in untreated patients within two years after their entry into the study [21]. It is also important to note that many authors indicate that, if patients develop renal insufficiency, this occurs with few exceptions within two to three years after presentation [17,18], and that prognosis in patients with normal renal function at five years after presentation is good. Because of this indolent course of idiopathic membranous glomerulonephritis and in view of the fact that the number of patients with complete or partial remission of proteinuria increases with time it would be important to know the long-term outcome [>10 years] of idiopathic membranous glomerulonephritis. Only few studies have thusfar been reported however [table III], and in only one, patients were untreated [25]. These studies confirm the idea that idiopathic membranous glomerulonephritis has a variable course, in the long run about 50% of patients reaching complete or partial remission and about 50% progressing to renal insufficiency. Persistent nephrotic syndrome after 10 years of follow-up is present in only 4-9% of patients [24,27]. The evolution to end-stage renal disease is rather slow, about 25-30% of patients being dialysis-dependent after a follow-up of 10 years. In the Japanese study a more favourable prognosis is given, a complete or partial remission of proteinuria being reported in about 70% of patients, only four out of 104 patients reaching ESRD after a mean follow-up of 11.5 years [26].

PROGNOSTIC FACTORS

From these and other studies a number of clinical and histological factors have emerged which determine the risk of developing renal insufficiency. An overview of these risks factors

Table IV. Prognostic factors in membranous glomerulonephritis

Sex (18,19,21,28,29)
Age (18,30,31)
Race (26)
Renal function at presentation (17,18)
Proteinuria (17,18,22,29)
Selectivity index (19)
Histological stage of glomerular lesions (25,29)
Tubulo-interstitial changes (17,21,24)
HLA-DR3, HLA-B18, BfF1 (5)

Number in brackets refer to the references

is given in table IV. We will address the importance of these factors briefly.

Sex. In general, membranous glomerulonephritis has a favourable course in women. Hopper and colleagues found a complete remission in 16 of 33 women [48%] as compared to 5 of 37 men [14%][28]. Likewise, in another study, a complete or partial remission occurred in 6 of 6 women as compared to 17 of 26 male patients [21]. Deterioration of renal function is less likely in women. Evidence of renal function deterioration was seen in 35% of women and 58% of men [19], and ESRD was reached in 10% of women after a mean follow-up of 88 months and in 39% of men after a mean follow-up of 66 months [29].

Age. Prognosis in children is rather good. In a study of 50 children, who where followed for one to 10 years [mean 4 years], 26 reached a complete remission and only five children progressed to renal failure [30]. When comparing the outcome in patients with onset of membranous glomerulonephritis before or after the age of 15 years Cameron reached a similar conclusion [31]. Remission of proteinuria was less likely in adults [16% vs 49%] and ESRD was more frequent [19% vs 4%]. Some authors feel that a somewhat better prognosis is also observed in adults under 30 years of age [18].

Race. Japanese patients apparently have a better prognosis, a complete or partial remission being reported in 70% of pa-

tients, only four out of 104 patients reaching ESRD after a mean follow-up of 11.5 years [26].

Renal function at presentation. In patients who present with renal insufficiency, renal function almost invariably shows a progressive decline. In the study of Davison, 70% of patients with a serum creatinine over 120 μmol/l at onset showed progression of renal failure as compared to 24% of patients with a serum creatinine below 100 μmol/l [18]. ESRD developed in three of seven patients with renal insufficiency as compared to eight of 109 patients without renal insufficiency [17].

Proteinuria. Patients with a nephrotic syndrome have a worse prognosis than patients with asymptomatic proteinuria. Evidence of progressive renal disease is found in 23% to 58% of nephrotic patients as compared to 7% to 25% of patients with asymptomatic proteinuria [17,18]. Donadio and colleagues calculated that after 10 years 5%, 18%, and 60% of patients with proteinuria of 0-3.4 g/24 h, 3.5-10 g/24 h, and >10 g/24 h respectively, had died or had reached ESRD [22]. Since the level of proteinuria determines serum albumin concentration it is not surprising that sarum albumin concentration correlates with the prognosis, 10 year survival approaching 76% in patients with albumin >15 g/l and 56% in patients with a serum albumin below 15 g/l [29].

Selectivity index. Glomerular proteinuria can be divided into a selective or a non-selective pattern based on a comparison of the clearance of albumin (or transferrin) with the clearance of larger molecular weight proteins (IgG). Based on the idea that proteinuria is simply the result of an increased diameter of the pores in the glomerular capillary wall, it was assumed that the selectivity index (defined as the clearance ratio of IgG: albumin (or transferrin) would reflect glomerular damage. Patients with a selectivity index less than 0.10 are defined as having highly selective proteinuria, and are considered to have

only a modest increase in glomerular permeability. In patients with membranous glomerulonephritis, a selectivity index below 0.20 is associated with a better prognosis. Coggins et al report a decrease of GFR of 10% per year in 33% of patients with selectivity index below 0.2 and in 60% of patients with a selectivity index above 0.2 [19].

Histological stage. Four pathologic stages have been described [1]. A stage I lesion includes small scattered subepithelial deposits associated with spike-like irregularities of the epithelial surface of the basement membrane. In stage II lesions, the deposits are more numerous, larger, and have a more uniform distribution throughout the glomerular tuft, and the epithelial spikes are prominent. In stage III the deposits become completely encircled by lateral extensions of the GBM spikes and are thus incorporated in the membrane. Finally, stage IV shows a definite rarefaction of the former deposits, together with an extremely variable thickening of the GBM. Although not confirmed in all studies some authors reported a better prognosis in stage I patients, survival being 85% in stage I patients as compared to 55% in stage III patients [29]. Zuchelli et al. reported a lower incidence of chronic renal failure in stage I patients (stage I: 0/12, stage II: 16/49, stage III-IV: 14/21)[25].

Tubulo-interstitial damage. Although the presence of tubulo-interstitial changes is associated with a poorer prognosis [17,21,24], quantitative data are scarce. Ponticelli et al described a relative risk of 3.4 [21].

HLA-DR linkage. A subgroup of patients with idiopathic membranous glomerulonephritis possesses the rare allotype of the complement component Bf, BfF1, in association with DR3 and B18. These patients apparently have a worse prognosis [5]. None of the patients reached a sustained remission of proteinuria, and three quarter developed renal insufficiency.

Corticosteroids and to a lesser extent other immunosuppressive drugs have been used in the treatment of membranous glomerulonephritis. Most studies were uncontrolled and have yielded equivocal results [9,10,15,32-35]. Furthermore, uncontrolled studies can hardly ever give definitive answers since the natural history of membranous glomerulonephritis is quite variable and dependent on many factors as discussed in the previous section. Only a few controlled studies are available for review [table V]. Older studies did not show any benefit of steroids [36], azathioprine [37,38], or cyclophosphamide [39]. However, since only a small number of patients, followed for a rather short period, were included, these studies do not permit to exclude any treatment-related benefits. Promising results were reported by a French group of investigators who treated patients with chlorambucil for 12 months. After a follow-up of two years, 13 of 16 chlorambucil-treated patients reached a complete or partial remission of proteinuria as compared to three of 14 placebo treated patients [40]. However, firm conclusions cannot be drawn from this study. First, no data on renal function and on deterioration of renal function were given. Second, since in untreated patients the percentage of patients reaching a remission increases with time, one cannot exclude that the difference in this respect between untreated and treated patients would eventually have disappeared. Finally, we are left with three large, fully documented controlled trials [19-21]. The first study came from the U.S. and was reported in 1979 [19]. Patients with idiopathic membranous glomerulonephritis and proteinuria of more than 3.5g/24h/1.73 m^2 were treated with 125-150 mg prednisone on alternate days for eight weeks. Follow-up averaged 23 months [range 4-52 months]. Although during follow-up complete and partial remissions occurred more frequently in treated patients [22/34 vs 11/38; $p<0.01$], at the time of the latest observation this difference was less striking: in the treated group four patients were in complete remission and eight in partial remission as compared

to four and three patients respectively in the placebo group. The main effect of treatment concerned the risk of progressive renal failure. Eleven of 38 placebo-treated patients experienced a doubling of serum creatinine during follow-up as compared to only two of 34 prednisone-treated patients. Although the methodology of this study seemed sound, some criticisms should be mentioned. Follow-up was rather short and the number of complete remissions was similar in both groups. Furthermore, the prognosis of the placebo group was exceptionally poor, rapid deterioration of renal function occurring in about 30% of patients. More recently the efficacy of prednisone treatment was addressed in a Canadian study [20]. In this study 81 patients were treated with 45 mg/m^2 prednisone on alternate days for six months, 77 untreated patients serving as controls. After a mean follow-up of 48 months no differences with respect to the occurrence of remissions of proteinuria or to the course of renal function could be demonstrated. Although this study appears to provide a strong case against any beneficial effects of prednisone treatment in idiopathic membranous glomerulonephritis, some important points need to be clarified. First, the treated [P] and control group differed with respect to several prognostic factors, e.g. serum creatinine [P: 120±10 µmol/l; control: 103±9 µmol/l; $p<0.05$], proteinuria [P: 6.9±0.8 g/24h; control: 5.2±0.9 g/24h; $p<0.05$], and percentage of female patients [P: 25%; control: 44%]. Furthermore, one quarter of patients did not have a nephrotic syndrome, and the prognosis of the control group was extremely good, 19 of 77 patients reaching a complete remission of proteinuria within three years, and only a minority of patients showing evidence of deteriorating renal function [25 % of patients had a more than 25% rise of serum creatinine at five years, and only 6% had reached ESRD at that time]. The last confounding factor may be the interval between the onset of disease and the start of treatment. Median known duration of disease at that time was 16 months in the Canadian study and six months in the U.S. study. Taken together this might suggest that institution of treatment at an early stage of the disease is more effective. The most

Table V. Controlled trials in membranous glomerulonephritis

Ref.	Treatment	Patient (No.)	Follow-up (years)	Proteinuria CR	Proteinuria PR	Deterioration of renal function
36	Prednisone	12	2	no exact data; overall no significant differences		
	Control	17				
37	Aza/Pred	5	0.5	no exact data; overall no significant differences		
	Control	9				
38	Azathioprine	5	1	0	ND	2
	Control	4		1	ND	3
39	Cyclophosphamide	11	1	0	4	stable
	Control	11		0	2	stable
40	Chlorambucil	16	2	9	4	ND
	Control	14		2	1	ND
19	Prednisone	34	2	4	8	2
	Control	38		4	3	11
20	Prednisone	81	4	16	24	24
	Control	77		19	19	21
21	Chl./Pred.	42	5	16	12	4
	Control	39		2	7	19

Abbreviations: Pred=prednisone; Aza=azathioprine; chl=chlorambucil; CR=complete remission; PR=partial remission; ND=no data

recent study came from Italy [21]. The investigators used chlorambucil and steroids in alternating monthly courses for six months. Patients were followed for an average of five years. Treated patients experienced significantly more complete and partial remissions of proteinuria [34/41 vs 13/39], which were sustained in most patients. Substantial deterioration of renal function occurred in only one of the treated patients, whereas 13 of 39 untreated patients showed a doubling of serum creatinine during follow-up. The results of this study are very promising, but await further confirmation.

Because of the short-term adverse effects and the potential long-term hazards of prednisone and other immunosuppressive drugs, it would be important to limit this treatment to

Table VI. Summary of uncontrolled therapeutic trials in patients with membranous glomerulonephritis and renal insufficiency[1].

Patients (No)	Sex (M/F)	Screat (μmol/l)	Therapy	Follow-up (months)	Proteinuria		Renal function			Ref.
					CR	PR	IM	S	ESRD	
8	7/1	194 (122-312)	Chl/P	18	1	3	6	1	1	41
7	7/0	300 (180-480)	Chl/P	11	1	1	1	3	0	42
15	13/2	410 (160-750)	P	32	0	2	9	1	5	43
9	7/2	222 (130-300)	C/P	33	4	5	4	2	0	44
9	7/2	266 (180-400)	P	98	3	3	7	2	0	45
10	6/4	297 (185-563)	Aza/P	33	2	3	6	2	1	46

[1]Only patients with deteriorating renal function are included.
Abbreviations: Screat=serumcreatinine, CR=complete remission, PR=partial remission, IM=improved, S=stabilised, ESRD=end stage renal disease, Chl=chlorambucil, P=prednisone, C=cyclophosphamide, Aza=azathioprine.

patients with progressive disease. To our knowledge thusfar no controlled trials have been conducted in patients with evidence of deteriorating renal function. Recently the results of several uncontrolled studies have been reported [41-46]. A summary of these studies is given in Table VI. Excellent results have been obtained by Mathieson et al, who treated eight patients with deteriorating renal function with chlorambucil and prednisone using exactly the same regime as Ponticelli including the three initial pulses of one gram of methylprednisolone. In all patients proteinuria decreased, one patient reaching a complete and three a partial remission. Renal function improved in six, and stabilised in one. Although in their original article follow-up was rather short averaging 11 months, the improvement proved to be sustained after a mean follow-up of 18 months [47]. From Table VI it is evident that beneficial effects in patients with membranous glomerulonephritis and deteriorating renal function have also been reported for long-term treatment with cyclophosphamide, azathioprine, and/or high dose prednisone. Overall, of 58 patients treated 11 reached a complete remission and 17 a partial remission of proteinuria. More importantly, in nearly three quarter of patients renal function improved or stabilized.

These results indicate that patients with membranous glomerulonephritis and deteriorating renal function may respond to several different immunosuppressive regimens. If the results of the study of Mathieson et al. are confirmed in further trials it would allow us to limit immunosuppressive therapy to patients with progressive disease. Treatment could then be withheld from patients in whom the disease follows an indolent course without progression to renal failure so that they will not be exposed to the short-term adverse effects and the potential long-term hazards of immunosuppressive drugs.

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CHAPTER III

INFLUENCE OF URINARY FLOW RATE ON PROTEIN EXCRETION IN PATIENTS WITH RENAL DISEASE

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ABSTRACT

We studied the influence of urinary flow rate on urinary protein excretion rate in nine patients with renal disease and proteinuria of over 1.5 g/24h. Our results demonstrate that urinary protein excretion is fairly constant and, at urinary flow rates higher than 1.5 ml/min, independent of diuresis. At lower urinary flow rates protein excretion is diminished, which may result from increased tubular reabsorption of proteins. In measuring protein excretion rates, urinary flow rate should be accounted for.

INTRODUCTION

Proteinuria is an important marker of renal disease. Correct quantitation of urinary protein excretion is important in the follow-up of patients, in interpreting therapeutic gains and in analysing investigational results. In most cases protein excretion is measured in 24 hour urine samples, but the values will vary according to differences in posture, exercise and diet. To circumvent this variability, especially when clinical investigations call for precise measurements, protein excretion rate is often measured using urine samples collected over a short period of time, the patient being supine during the investigation. However, urinary flow rate is seldom accounted for.

There is no consensus regarding the influence of urinary flow rate on the excretion of proteins [1]. The results of the few studies on this subject are conflicting [2-6]. Furthermore, all studies have been performed in normal or diabetic subjects with normal or almost normal protein excretion rates. Therefore, we have studied the influence of urinary flow rate on protein excretion in patients with renal disease and frank proteinuria. Our results show that flow rate may influence protein excretion at flow rates under 1.0 ml/min, whereas there is no such influence at flow rates higher than 1.5 ml/min.

PATIENTS AND METHODS

We studied nine patients, with a mean age of 42 years (range 18-69 years) and a mean endogenous creatinine clearance of 84 ml/min (range 16-125 ml/min). All had proteinuria of over 1.5 g/24 h. The clinical data are given in table I.

In each patient a number of consecutive urine samples (mean 8; range 4-15) were collected during short intervals of 40-120 min either by spontaneous voiding (in eight patients) or an indwelling bladder catheter (patient no.1). Patients were supine during the investigation, except during voiding, when they were

Table I. Clinical data

Patient no.	Sex	Age	Diagnosis	ECC (ml/min)	Medication
1	M	24	MGN	75	Prednisone
2	F	18	FGS/RT	89	Prednisone, Ciclosporine
3	F	50	FGS	16	Atenolol, Nifedipine, Bumetanide
4	M	55	ML	115	Acenocoumarol
5	M	34	RT rejection	80	Prednisone, Azathioprine, Atenolol, Apresoline
6	M	57	MGN	80	Atenolol
7	F	49	FGS	125	-
8	F	22	MGN	117	-
9	F	69	MGN	56	Atenolol, Chlorthalidone, Captopril

Abbrevations: ECC=Endogenous creatinine clearance; M=Male; F=Female; MGN=Membranous glomerulonephritis; FGS=Focal glomerular sclerosis;RT=Renal transplantation; ML=Minimal lesions glomerulonephritis.

in an upright or sitting position to ensure complete emptying of the bladder. Fluid intake was moderate in order to avoid negative effects of excessive hydration or rapid fluctuations of urinary flow rate. Salt intake was not controlled. During the day of the investigation protein rich meals were not allowed, because of their known influence on glomerular filtration rate [7].

Urine collection periods were exactly timed, urine volume was measured, and concentrations of creatinine and protein were measured in all urine samples. In patient 1, glomerular filtration rate was measured simultaneously using Inutest (Polyfruc-

tosan, Laevosan Gesellschaft, Linz, Austria) as a marker for glomerular filtration. Creatinine, protein, and inulin were measured using standard colorimetric methods.

Spearman's rank correlation coefficients were computed and used for statistical analysis.

RESULTS

The influence of urinary flow rate on protein excretion rate is shown in figures 1 and 2. In three patients (2,3, and 5) flow rates above 1.2 ml/min were not achieved. Two of these patients had severe proteinuria (maximum protein excretion rate > 8 mg/min). In three patients (4,6, and 7), in whom both low and

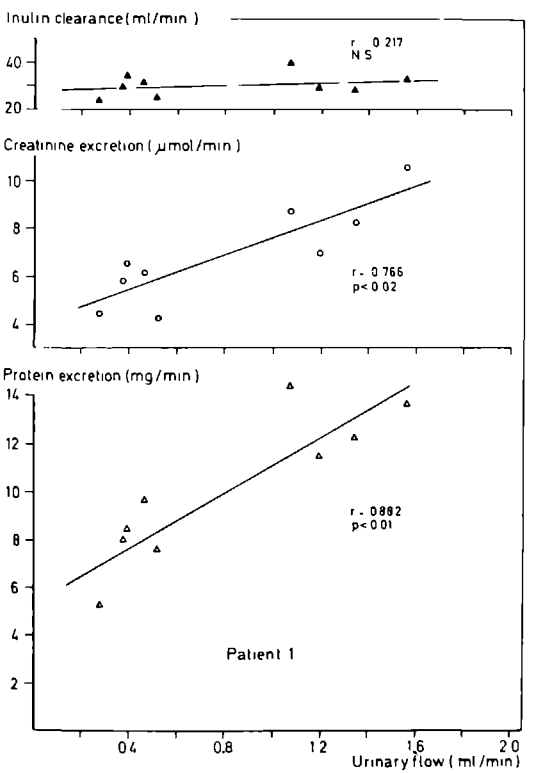

Figure 1.
Relationship of excretion rates (protein, creatinine) and inulin clearance with urinary flow rate in patient 1.

high flow rates were achieved, the plots of urinary flow rate versus urinary protein excretion rate could be resolved in two linear parts by visual fit. The results show that protein excretion remains fairly constant at flow rates higher than 1.0-1.5 ml/min. At lower urinary flow rates protein excretion is reduced. Similar observations were made for creatinine excretion. Figure 1 shows that these changes in urinary protein and creatinine excretion in patient 1 were not accompanied by a change in inulin clearance, suggesting that glomerular filtration rate remained constant.

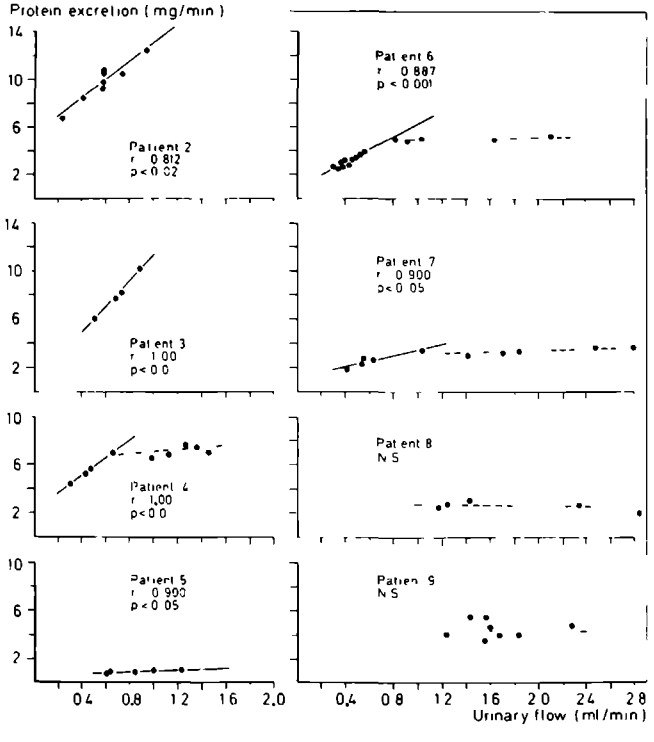

Figure 2.
Relationship between protein excretion and urinary flow rate in patients 2-9. The correlation coefficients (r) refer to the solid lines. Broken lines represent a non-significant correlation.

Our study demonstrates that at least in patients with overt proteinuria urinary protein excretion is fairly constant and independent of diuresis at urinary flow rates above 1.5 ml/min. At lower urinary flow rates, a possible relation between protein excretion and urinary flow rate seems to exist.

To our knowledge, the influence of urinary flow on urinary protein excretion rate has only been studied in normal individuals or diabetic patients with a protein excretion rate in the normal range [2-6]. In three studies a correlation of urinary flow with urinary protein excretion rate was demonstrated [2,3,6]. However, firm conclusions cannot be drawn from these studies. A possible influence of posture was not accounted for in one study [2]. In the two other studies, urinary flow was increased by excessive water loading [3,6], which causes a short-lived enhancement of protein excretion ascribed to an increase of glomerular filtration rate [4]. Lastly, rapid changes in urinary flow rate were accepted in all studies, which could have biased the results in view of the dead space of the tubulo-pelvi-ureteral system. In rat studies, an influence of tubular flow rate on the excretion of horseradish peroxidase (HRP) was found [8]. Injection of HRP, however, caused a considerable decline of GFR and an increase of vascular permeability. Therefore, these results are also difficult to interpret.

That proteinuria is independent of diuresis can easily be explained since proteins are reabsorbed in the proximal tubule [9], whereas changes in urine flow are predominantly modulated in the distal and collecting tubules. We found only an influence of urinary flow rate on proteinuria at flow rates below 1.0-1.5 ml/min. As indicated by the inulin clearance in patient 1, it is unlikely that changes in glomerular filtration rate are responsible for this effect. Although it is difficult to draw firm conclusions from observations in only one patient,

this conclusion is strengthened by the fact that in mammals variations in urinary flow rate rarely reflect variations in renal blood flow, or glomerular filtration rate, unless urinary flow is below 0.35 ml/min [10,11]. Indeed, in man and dogs no correlation of glomerular filtration rate with urinary flow rate was found at flow rates above 0.6 ml/min [12,13]. The decrease of urinary protein excretion at low urinary flow rates might result from an increased tubular reabsorption of proteins. In such a case, one must assume that at flow rates between 0.5-1.5 ml/min changes in proximal tubular fluid reabsorption must occur, probably caused by small changes in extracellular volume, which can directly influence proximal tubular fluid reabsorption at constant filtration rates [14]. It remains difficult to exclude, however, that the decreased protein excretion and its association with a decreased urinary flow rate is an artefact, caused by incomplete emptying of the bladder, or the sequestration of urinary protein in the urinary dead space.

Our findings that creatinine excretion is flow dependent agree with those of others [15]. Whether this effect of urinary flow rate on creatinine excretion is an artefact as mentioned above, or can be explained by flow dependent tubular secretion, or as the result of enhanced creatinine reabsorption in the bladder at low flow rates [16], cannot be determined from our data.

Despite these uncertainties our findings have practical implications. Our study demonstrates that at least in patients with renal disease and proteinuria, the protein excretion rate is fairly constant at urinary flow rates above 1.5 ml/min. At very low flow rates, proteinuria is underestimated. Therefore, when studying these patients urinary flow rate should be accounted for.

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CHAPTER IV

RENAL CLEARANCE OF PANCREATIC AND SALIVARY AMYLASE RELATIVE TO CREATININE CLEARANCE IN PATIENTS WITH RENAL DISEASE AND PROTEINURIA

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Renal Clearance of Pancreatic and Salivary Amylase Relative to Creatinine Clearance in Patients with Renal Disease and Proteinuria

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To study the charge-selective properties of the glomerular filter in renal disease, we measured the fractional clearance relative to creatinine clearance (ECC), of the amylase isoenzymes pancreatic amylase and salivary amylase, which have identical size but different charge In 63 healthy subjects the mean (and SD) fractional excretion of pancreatic amylase 4 07% (1 24%), was fourfold that of salivary amylase 1 02% (0 54%) For 29 patients with renal disease and proteinuria, the mean fractional excretion of pancreatic amylase was significantly lower, 3 31% (1 94%), and that of salivary amylase significantly higher, 2 06% (1 41%), than in controls In these patients, fractional excretions of both these isoenzymes were negatively correlated with urinary excretion of β_2-microglobulin and ECC Evidently, differences in clearances of pancreatic and salivary amylase are a consequence of differences in charge-related glomerular filtration The relative increase of salivary amylase clearance in patients with renal disease and proteinuria is most probably caused by a loss of the charge-selective properties of the glomerular basement membrane

Human serum amylase (1,4-α-D-glucanglucanohydrolase, EC 3 2 1 1) consists of two major isomers, pancreatic amylase (P-amylase) and salivary amylase (S-amylase), which have identical size (2 9 nm), but different charge, S-amylase (isoelectric point 5 9–6 4) being more anionic than P-amylase (isoelectric point 7 0) (*1, 2*) The urinary excretion of amylase is governed by glomerular filtration and tubular reabsorption (*3, 4*) It has gradually become evident that transport of macromolecules through the glomerular filter is determined not only by the size of the molecule, but also to an important degree by its charge (*5–7*) The negatively charged glomerular basement membrane impairs filtration of anionic proteins such as albumin Because of the differences in charge of P- and S-amylase, one might expect important differences in renal clearance of these isoenzymes However, differences in renal processing of amylase isoenzymes have received little attention Therefore, we measured the renal clearance of amylase isoenzymes in patients with renal disease and proteinuria and compared the results with our values for healthy volunteers Our results show a preferential increase of clearance of S-amylase over P-amylase in patients with renal disease and proteinuria, pointing to a defect in the charge-selective characteristics of the glomerular basement membrane

Patients and Methods

Patients Amylase clearances were measured in 63 healthy controls (group I) and in 29 patients with renal disease and proteinuria (group II)

Group I comprised 34 men and 29 women with a mean (± SD) age of 39 ± 18 years (range 18–85 y) All volunteers had normal renal function (mean serum creatinine 78 ± 10 μmol/L), and no evidence of any underlying disease

Group II comprised 19 men and 10 women, with a mean age of 43 ± 16 years (range 17–67 y), a mean serum creatinine of 165 ± 107 μmol/L, a mean creatinine clearance of 78 ± 42 mL/min, and mean proteinuria of 7 0 ± 3 7 g/24 h (range 0 5–14 5 g/24 h) The underlying renal disease was glomerular in nature in all but one of the patients membranous glomerulonephritis (n = 11), focal glomerulosclerosis (n = 6), minimal change glomerulonephritis (n = 4), IgA nephropathy (n = 4), mesangiocapillary glomerulonephritis (n = 1), amyloidosis (n = 1), Alport hereditary nephritis (n = 1), and pyelonephritis (n = 1) Informed consent was obtained from all volunteers and patients

Clearance protocol Results of pilot experiments showed that both amylase isoenzymes could be accurately measured only if the urinary pH was between 6 8 and 7 2 Therefore the subjects were administered 4 g of sodium bicarbonate orally on the evening before the study and another 2 g in the morning at 2 h before urine collection All subjects were asked to drink 500 mL of tap water, to promote diuresis Thereafter a 2-h urine specimen was collected in 10 mL of phosphate buffer (1 0 *mol/L*, pH 7 0) Blood was sampled in the middle of this 2-h interval

Determinations We measured catalytic activity concentrations of total amylase and its isoenzymes, using the "Blue Starch" method (Phadebas, Pharmacia, Uppsala, Sweden), at 37 °C To all urine samples we added 1 mg of bovine serum albumin per milliliter All measurements were done in unfrozen samples within 48 h after collection We determined P- and S-amylase by the method of O'Donnell et al , using an S-amylase inhibitor (*8*) Creatinine in serum and urine was determined by the Jaffé technique (*9*) Proteinuria was measured by the biuret method In 22 of the patients we concurrently measured the urinary excretion of β_2 microglobulin by radioimmunoassay (β_2M-RIA, Pharmacia, upper limit in healthy subjects 0 15 μg/min)

Calculations Clearances were calculated by the usual formula

$$\text{clearance}_x = (U_x \cdot V/P_x)$$

where U_x is the concentration of substance x in urine, V is the urine flow rate, and P_x is the concentration of substance x in the plasma

Clearance of creatinine was used as marker of glomerular filtration rate Fractional excretions of amylase and its isoenzymes were calculated as clearance of amylase divided by creatinine clearance, and expressed as percentages

Statistical analysis We used the Wilcoxon test for unpaired observations Correlation was calculated according to Spearman A *P*-value of <0 05 was considered significant Unless otherwise mentioned, all values are given as means ± SD In cases of nonparametric distribution, median values are also given

Results

Table 1 gives values for total amylase and its isoenzymes in serum and for fractional excretions Two of the healthy volunteers showed no S-amylase in serum, and in a further 11 no S-amylase was found in urine Therefore, fractional

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Table 1. Results of Amylase Measurements in Control Subjects and Patients with Renal Disease

	Control subjects (n = 63)	Patients (n = 29)	P-value
Serum			
Total amylase, U/L	201 ± 49	238 ± 93	NS
P-amylase, U/L	98 ± 34	126 ± 63	$P < 0.05$
S-amylase, U/L	105 ± 48[a]	112 ± 77	NS
FE_{Tamy}, %	2 43 ± 0 74	2 71 ± 1 63	NS
FE_{Pamy}, %	4 07 ± 1 24	3 31 ± 1 94	$P < 0.01$
FE_{Samy}, %	1 02 ± 0 54[b]	2 06 ± 1 41[c]	$P < 0.001$
P/S ratio (median)	6 58 ± 8 02[b] (3 53)	2.09 ± 1 38[c] (1 60)	$P < 0.001$

Abbreviations P = pancreatic, S = salivary, FE_{Tamy} = fractional excretion of total amylase, FE_{Pamy} = fractional excretion of pancreatic amylase, FE_{Samy} = fractional excretion of salivary amylase, P/S ratio = FE_{Pamy}/FE_{Samy}, NS, not significant [a]n = 61, [b]n = 50, [c]n = 28

excretions of S-amylase could only be determined in 50 of the 63 normal controls. By contrast, urinary S-amylase could not be demonstrated in only one of the 29 patients. When we compared the 50 individuals with detectable urinary S-amylase and the 11 individuals with undetectable urinary S-amylase we found a significant difference in serum isoenzyme pattern. The percentage of P-amylase in serum was 47.0 ± 15 8% in the former and 62.8 ± 12 5% in the latter group ($P < 0.01$), whereas results for total amylase in serum were not significantly different (187 ± 60 and 206 ± 54 U/L, respectively).

It is evident from Table 1 that, in the patients with renal disease, the fractional excretion of P-amylase was significantly less than in controls, whereas the fractional excretion of S-amylase was significantly greater. As a result the ratio of fractional excretion of P-amylase to fractional excretion of S-amylase (P/S ratio) was significantly lower, with a median value of 1.60 (range 0 66–6.71), as compared with 3 53 (range 1.79–49.5) for the normal controls ($P < 0.001$) In the patients, fractional excretion of amylase and amylase isoenzymes correlated significantly with proteinuria (FE_{Tamy} vs proteinuria: $r = 0.49$, $P < 0.02$, FE_{Pamy} vs proteinuria $r = 0.53$, $P < 0.01$, FE_{Samy} vs proteinuria: $r = 0\ 54$; $P < 0.01$).

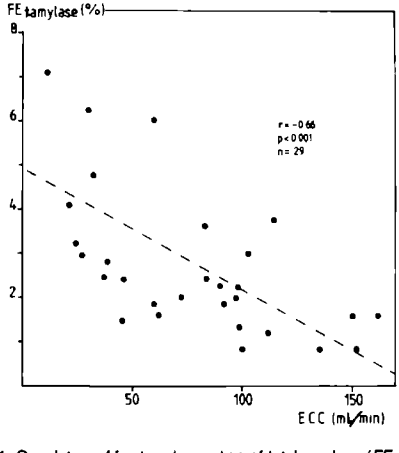

Fig 1 Correlation of fractional excretion of total amylase ($FE_{T\text{-}amylase}$) with endogenous creatinine clearance (*ECC*) in patients

Fractional excretion of total amylase correlated significantly with creatinine clearance (Figure 1).

The fractional excretion of amylase isoenzymes similarly correlated with creatinine clearance (FE_{Samy} vs ECC: $r = -0.48$; $P < 0.01$; FE_{Pamy} vs ECC: $r = -0\ 67$, $P < 0\ 001$) In the 22 patients in whom we measured the urinary excretion of β_2-microglobulin ($U\beta_2M$), we found a significant correlation of this rate with creatinine clearance ($r = -0\ 69$; $P < 0.001$) In these patients the fractional excretion of total amylase and amylase isoenzymes also correlated significantly with urinary β_2M excretion (FE_{Tamy} vs $U\beta_2M$ $r = 0\ 60$; $P < 0\ 01$, FE_{Samy} vs $U\beta_2M$. $r = 0.59$, $P < 0\ 01$, FE_{Pamy} vs $U\beta_2M$: $r = 0\ 67$, $P < 0\ 001$) The patients could be divided according to β_2-microglobulin excretion rate (Table 2). In nine patients β_2M excretion was normal or only slightly increased (<1.5 μg/min; mean 0 36 ± 0 13 μg/min) In the others, β_2M excretion clearly exceeded 1 5 μg/min (mean 22 5 ± 19 6 μg/min) On comparing these groups of patients we observed a significantly lower creatinine clearance accompanied by significantly increased fractional excretions of total amylase, P-amylase, and S-amylase in the group of patients with the high urinary β_2M excretion. The P/S ratio was not different, however. When we categorized patients according to their original renal disease, the P/S ratio was lowest in patients with minimal-lesions glomerulonephritis (P/S ratio = 1 37 ± 0.26; n = 4), as compared with 2 00 ± 0 44, 2.25 ± 0.91, and 2.67 ± 0.43 in patients with membranous glomerulonephritis (n = 11), focal glomerulosclerosis (n = 6), and proliferative glomerulonephritis (n = 5), respectively.

Discussion

For the normal volunteers the fractional clearance of total amylase averaged 2.4%, a value similar to those reported in the literature, which range from 1 24 to 3 1% (*1, 10–16*). Fractional excretion of P-amylase was 4 07%, a value higher than those reported in the literature, which range from 1 75 to 3 5% (*11, 12, 14, 17–19*) The fractional excretion of P-amylase exceeded that of S-amylase by three- to fourfold This preferential loss of P-amylase in control subjects has already been reported. ratios of the fractional excretion of P-amylase and fractional excretion of S-amylase reportedly range from 1 6 to 6 (*11, 12, 14, 16–21*) The differences in the results of these studies can partly be explained by methodological differences, several different techniques having been used to determine amylase isoenzymes, e.g , cellulose acetate electrophoresis (*12*), diethylaminoethyl ion-exchange chromatography (*14, 21*), polyacrylamide gel electrophoresis (*16*), and thin-layer isoelectric focussing (*17, 18*). However, O'Donnell and coworkers (*11, 19*) found values of 2.64% and 1.64% for the fractional excretion of P- and S-

Table 2. Results of Amylase Measurements in Patients with Renal Disease in Relation to β_2-Microglobulin ($U\beta_2M$) Excretion Rate

	$U\beta_2M$ <1 5 μg/min (n = 9)	$U\beta_2M$ >1.5 μg/min (n = 13)	P-value
ECC, mL/min	114 ± 29	59 ± 33	$P < 0\ 01$
FE_{Tamy}, %	1 55 ± 0 63	3 44 ± 1 76	$P < 0\ 01$
FE_{Pamy}, %	1 96 ± 0 74	4 27 ± 2 09	$P < 0\ 01$
FE_{Samy}, %	1 18 ± 0 67	2 62 ± 1 60	$P < 0\ 02$
P/S ratio (median)	2 30 ± 1 84 (2 07)	2 06 ± 1 37 (1 56)	NS

Abbreviations ECC, endogenous creatinine clearance For other abbreviations, see Table 1

amylase, respectively, and they used an inhibitor technique similar to ours We cannot easily explain the differences between results of their and our study, but two points need to be considered First, they determined amylase in serum and urine samples that had been stored at −20 °C for five days Second, for their control subjects they found values for P-amylase in serum (averaging 65% of total amylase) that clearly exceeded values reported by others, which range from 40 to 50% (*10, 12, 14, 16, 17, 21–24*)

Some of these authors also used an inhibitor technique (*23, 24*) It can be expected that in the subjects of O'Donnell et al the proportion of P-amylase in urine as compared to total amylase would easily exceed 90% At this high percentage of urinary P-amylase the inhibitor method is insensitive (*25*) and measurements will give falsely high values for S-amylase (*8*) In this respect, it is important to note that, in our study, fractional excretion of both amylase isoenzymes could be determined in only 50 of the 63 volunteers (79%), and in 11 subjects we could detect no S-amylase in urine In these subjects, serum P-amylase (expressed as percentage of total amylase) was significantly higher In view of the higher clearance of P-amylase it can be calculated that the percentage of P-amylase relative to total amylase in the urine will exceed 90% in these subjects

In the patients with renal disease and proteinuria, fractional excretion of P-amylase was decreased and that of S-amylase was increased as compared with control values In the patients, the fractional excretions of amylase and amylase isoenzymes were negatively correlated with ECC, in agreement with others (*10, 12, 13, 15, 19*) This finding can be explained by the decrease of tubular reabsorptive capacity as renal insufficiency progresses Comparing patients with normal and abnormal tubular function, we observed no differences in the P/S ratio This indicates that both isoenzymes are processed by the glomerular tubules in the same way Therefore, the current opinion of most authors that the differences in urinary excretion of P-amylase and S-amylase are a consequence of difference in tubular reabsorption is not supported by our results

The restricted transport of S-amylase must be attributed to its negative charge, which is consistent with recent observations that the net electric charge on a molecule is an important determinant of its fractional clearance (*6, 7*) The decreased fractional excretion of P-amylase in patients with proteinuria fits well with recent findings that the transglomerular transport of neutral dextrans of 2 0–4 6 nm size is restricted in patients with nephrotic syndrome (*26*) The relative increase of fractional excretion of S-amylase points to a defect in the charge selectivity of the glomerular capillary wall Such a defect has been demonstrated in several experimental models of glomerulonephritis and in humans with diabetes mellitus, congenital nephrotic syndrome, and minimal-lesions glomerulonephritis (*6*) In agreement with the latter observations, the P/S ratio was lowest in the patients with minimal-lesions glomerulonephritis It seems worthwhile to study further the possible usefulness of fractional excretions of P- and S-amylase as markers of glomerular basement membrane charge

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CHAPTER V

CREATININE AS A MARKER OF GLOMERULAR FILTRATION RATE

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Review

Creatinine as a marker of glomerular filtration rate

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Creatinine is the most widely used marker of glomerular filtration rate in general clinical practice. The reciprocal of serum creatinine can be used to examine the time course of glomerular filtration rate. We review normal production and excretion of creatinine and discuss factors which may invalidate the use of creatinine as a marker of glomerular filtration rate. Neth J Med 1988;33:144–153.

Key words Creatine, Creatinine, Renal function, Glomerular filtration rate

Introduction

The glomerular filtration rate (GFR) is an important parameter of renal function Theoretically, GFR can be measured reliably by determining the renal clearance of substances which meet with the following criteria completely filterable at the glomerulus, no binding to plasma proteins, absence of metabolism, no tubular reabsorption or secretion [1] Examples of markers used for measurement of GFR are inulin, [^{51}Cr]EDTA (ethylenediaminetetraacetic acid), and [^{99}Tc]DTPA (diethylenetriaminepentaacetic acid) However, clearance measurements using these exogenous substances are impractical in routine clinical practice because of the need for intravenous administration, the collection of multiple blood (and sometimes urine) samples, the use of radioactive materials, or the involvement of time consuming laboratory techniques Since creatinine has none of these disadvantages, and largely satisfies the above-mentioned criteria, it is not surprising that it is still widely used as a marker of GFR In recent years, the reciprocal of serum creatinine (1/Screat) has become popular for examining changes in renal function in time and for assessing the possible effects of therapeutic interventions [2,3] However, as will be discussed below, several pitfalls exist in the use of creatinine as a marker of GFR

Production and Clearance of Creatinine

The creatinine precursor creatine is derived from three amino acids arginine, glycine, and S-adenosylmethionine [4] Two enzymatic steps are involved in the formation of creatine, which primarily takes place in the kidney, liver, and pancreas. Creatine is selectively incorporated in muscle and partly phosphorylated to phosphocreatine (Fig 1) This phosphocreatine is an important source of phosphate, used for the regeneration of adenosine triphosphate (ATP) after muscle contraction Since 90–98% of body creatine is stored in muscle, and its concentration in muscle is relatively fixed, the total body pool of creatine is relatively constant and dependent on total muscle mass Creatine and phosphocreatine are non-enzymatically dehydrated to creatinine at a constant rate, averaging 1.7%/24 h [4,5]. Since creatinine has a low molecular weight (MW 113), and is not bound to plasma proteins, it is completely filterable at the glomerulus. Under normal circumstances, metabolism is negligible while tubular transport contributes less than 20% to overall creatinine clearance [6,7]. Thus, GFR can be estimated by determining creatinine in blood and in a timed urine sample and by calculating the renal clearance of endogenous creatinine (ECC) according to the formula:

$$ECC = \frac{Ucreat}{Screat} \times V,$$

where Ucreat = concentration of creatinine in urine; Screat = concentration of creatinine in serum; V = urine flow rate (ml/min)

As mentioned above, the production and consequently the urinary excretion of creatinine are relatively constant and largely depend on muscle mass and thus on sex, body weight, and age [8,9]. In recent years, formulae have been developed for the estimation of ECC in adults from serum creatinine, sex, body weight, and age

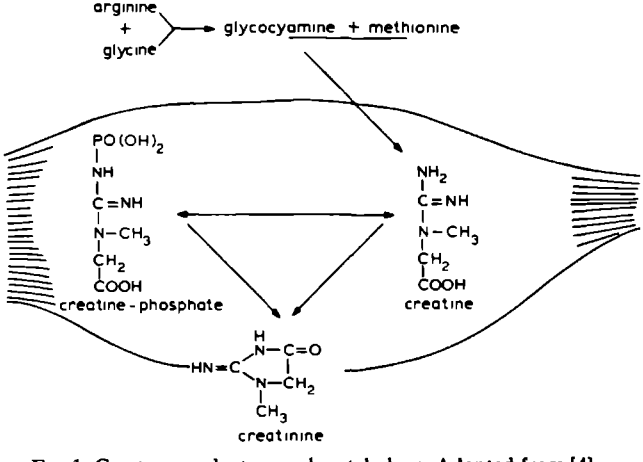

Fig 1 Creatine production and metabolism Adapted from [4]

TABLE 1

Formulae for estimation of ECC in adults

Males	Females	Ref
$\frac{(140 - \text{age}) \times \text{body weight}}{0.81 \times \text{Screat}}$	$\times 0.85$	10
$X + (55\text{-age})\ (0.005)\ X$ $X = 0.413\ \text{Screat}^{-1.2}$	$Y + (56\text{-age})\ (0.005)\ Y$ $Y = 0.434\ \text{Screat}^{-1.1}$	8
$98 - 16\frac{(\text{age} - 20)}{20}$ $0.0113 \times \text{Screat}$	$\times 0.90$	11

Age years body weight kg serum creatinine (Screat) μmol/l

[8,10,11] In the studies mentioned in Table 1, the estimated values of ECC correspond well with measured ECC However, the prediction error (expressed as a percentage of ECC) is considerable, exceeding 20% in one-third of the patients [8] Furthermore, in patients with liver disease calculated values overestimate measured values [12], probably as a consequence of a decrease in creatine or creatinine production in these patients In a given patient, creatinine excretion (Ucreat $\times$ V) can be considered constant in time Therefore, changes in ECC will be paralleled by changes in the reciprocal of serum creatinine (1/Screat) This ratio is now widely used to follow renal function in time [2,3] In patients with progressive renal insufficiency, 1/Screat has been shown to decrease linearly with time [13], allowing us to predict the time of end-stage renal failure The time course of 1/Screat is also being used to assess the effects of therapeutic interventions on the progression of renal insufficiency [2,3] It is important to note that the above-mentioned calculations and estimations are restricted to situations with only slow changes in renal function, where production and excretion of creatinine are in balance However, in cases where renal function changes rapidly, such as acute renal failure, serum creatinine can easily be used to give quantitative information on the course of renal function impairment [14]

Pitfalls involved in the use of creatinine as a marker of GFR

A survey of confounding factors influencing serum creatinine and urinary creatinine excretion is given in Table 2 The most important factors will be discussed below

Creatine and creatinine derived from exogenous sources Creatine and creatinine are not only derived from endogenous production Meat is an important dietary source of creatine [15] This creatine is partly converted to creatinine by heating, a process which is dependent on the duration of heating and the temperature reached (Table 3) Eating of boiled or baked meat can cause up to a two-fold increase of serum creatinine 2 to 3 h later [16] Apart from these acute effects, dietary meat content can also influence long-term creatinine excretion by changing the total body

TABLE 2

Factors that impair the reliability of serum creatinine or creatinine clearance as a precise measure of glomerular filtration *.

Factor	Magnitude of effect (%)	Cause	Influence on		
			Screat	ECC	1/Screat
Strenuous exercise	5– 10	A	↑	×	?
Dietary protein intake					
(high protein)	10– 30	A	↑	×	–
		B	↓	+	+
(low protein)	10– 30	A	↓	×	+
Renal disease	20–100	B	↓	+	+
		D	↓	×	+
Severe infection, fever, trauma	20–100	A	↑	×	–
Drugs					
trimethoprim, cimetidine	20	B	↑	–	–
Acetoacetate	0–100	C	↑	–	–
Muscular atrophy	0–100	A	↓	×	+

Screat = serum creatinine; ECC = endogenous creatinine clearance; A = increased/decreased production of creatinine from endogenous or exogenous sources; B = increased/decreased tubular secretion of creatinine, C = interference with creatinine determination; D = increased extrarenal clearance of creatinine; ↑ = increase; ↓ = decrease; + = overestimation of glomerular filtration rate; – = underestimation of glomerular filtration rate, × = unchanged

* For references see text.

creatine pool. An increase or reduction in dietary meat intake of 100 g/day, equalling 450 mg of creatine, will alter urinary creatinine excretion by 3.5 mmol/day or approximately 25% of normal creatinine excretion. Because of the slow turnover rate of creatine, changes in dietary creatine content will lead to gradual changes in urinary creatinine excretion. This has been demonstrated by Bleiler and Schedl in healthy volunteers [17]: after introduction of a creatine-free diet, urinary creatinine excretion decreased by 30–40%. A new steady state was reached only after 6 to 8

TABLE 3

Creatine and creatinine content of different meats prepared by different methods *

Meat	Method of cooking	Creatine content (mmol/100 g)	Creatinine content (mmol/100 g)
Round steak	Uncooked	3.74	0 371
	Boiled in water		1.36
	Baked in moist heat		1.77
	Baked in dry heat	0 31	2 86
Lamb	Baked in moist heat		1 51
Perch	Baked in moist heat		0.66

* According to Camara et al [15].

wk Also, a parallel decrease of serum creatinine was noted from a mean value of 120 μmol/l to 90 μmol/l Similarly, in patients with renal insufficiency, the introduction of a low protein diet (0 2 g protein/kg body weight/24 h) caused a decrease of serum creatinine from 930 ± 341 μmol/l to 688 ± 324 μmol/l (mean ± SD) [18] This decrease of serum creatinine concentration was paralleled by a decrease of urinary creatinine excretion, implying that ECC remained unchanged Use of the reciprocal of serum creatinine as an indicator for FCC would have suggested an amelioration of renal function It is important to consider these dietary effects when using 1/Screat as an equivalent of ECC Recent studies have claimed beneficial effects of early protein restriction on the progression of renal disease [2,3] In these studies 1/Screat has been used to evaluate renal function Reduction of meat intake per se would be expected to increase 1/Screat thus suggesting an amelioration of renal function Indeed, in their study, Mitch et al [2] have demonstrated that in the subgroup of their patients, in whom creatinine excretion was also measured, 1/Screat suggested an improvement of renal function, whereas the measured ECC actually decreased

Tubular handling of creatinine In man, there is clear evidence of tubular secretion of creatinine [19] Under normal circumstances, up to 20–30% of creatinine excretion is caused by tubular secretion [7], leading to a ratio of ECC/GFR of 1 2–1 3 Transport of creatinine is an active, saturable process, probably located in the proximal tubule [19,20] Tubular secretion can be inhibited by both anionic and cationic substances [21–26] The latter group is clinically important because it includes frequently used drugs such as trimethoprim [22,23], cimetidine [24 25], and salicylates [26] Administration of these drugs will lead to an increase of serum creatinine, suggesting a decline of renal function, while measurement of GFR reveals no changes Tubular secretion of creatinine can also be stimulated Administration of creatinine has been demonstrated to enhance its own tubular secretion [19] In line with this, we recently found changes of tubular creatinine secretion in patients with renal disease when the protein intake was modified In 8 patients who adhered to a high protein diet for 4 wk and were then switched to a low protein diet for another 4 wk, GFR (inulin clearance) did not change significantly However, the ratio ECC/GFR which can be used as a measure of tubular creatinine secretion decreased significantly from 1 60 ± 0 04 (mean ± SEM) on the high protein diet to 1 48 ± 0 04 on the low protein diet ($P < 0\ 05$, Fig 2) Similarly, tubular secretion of creatinine is increased in patients with renal insufficiency [27] This partly offsets the expected rise in serum creatinine when GFR decreases, so that a significant rise in serum creatinine is not observed before GFR decreases below 60 ml/min [27] Furthermore, ECC will overestimate GFR more as renal insufficiency progresses (Fig 3). Some authors claim that tubular secretion of creatinine is even more important in patients with a nephrotic syndrome [28], but this is denied by others [29,30] Changes in tubular secretion may explain why small changes in GFR will be undetectable when using ECC [3] Although tubular reabsorption of creatinine has been demonstrated in rats, and has been suggested by experiments in sheep, goats, and dogs [20,32], in man only circumstantial evidence points to the possibility of

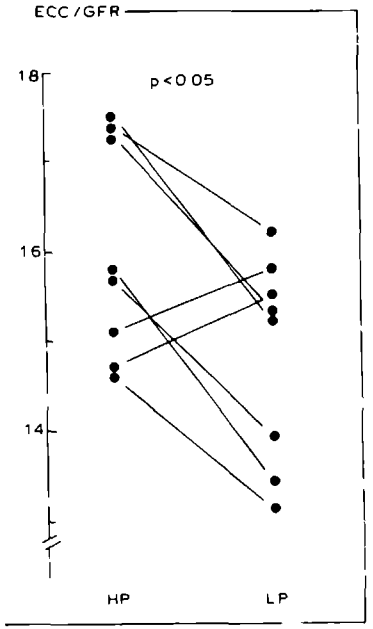

Fig 2 Ratio ECC/GFR in patients with renal disease on a high protein diet (HP) and a low protein diet (LP) (Personal observations GFR was measured using inulin clearance Creatinine was measured using alkaline picrate)

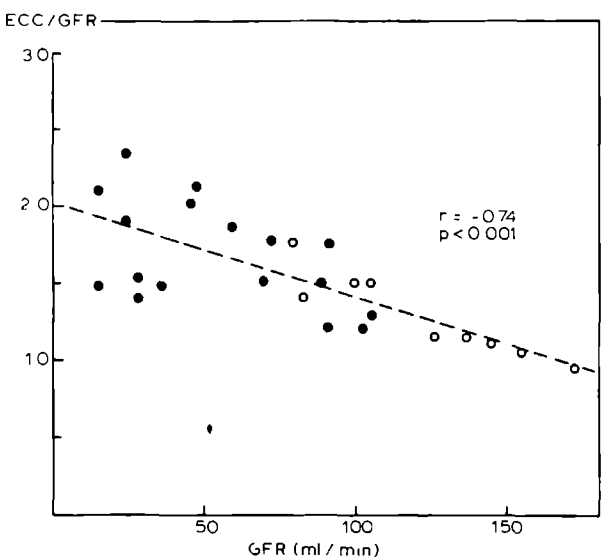

Fig 3 Ratio ECC/GFR in relation to renal function (GFR) ECC overestimates GFR more as renal insufficiency progresses (Personal observations GFR was measured using inulin clearance in healthy volunteers (○) and patients with renal disease (●) Creatinine was measured using alkaline picrate)

TABLE 4

Substances interfering with the determination of creatinine using alkaline picrate.

Acetoacetate [38,43]
Oxaloacetate [43]
Pyruvate [43]
Cephalosporins [45,46]
Non-creatinine chromogens [7]
Methyldopa [47]
Bilirubin [42]

Numbers in brackets refer to the references.

tubular reabsorption of creatinine [33,34]. Further studies are needed regarding this subject.

Metabolism of creatinine. In healthy volunteers and patients with normal renal function, metabolism of creatinine is negligible, over 98% of creatinine being recovered in the urine [6]. However, in patients with renal insufficiency, more substantial metabolism of creatinine has been demonstrated [6,35]. The degradation rate is correlated with the serum creatinine concentration, and in patients approaching end stage renal failure, up to 65% of creatinine may be metabolized, probably in the gut [35]. This explains the decrease of 24 h urinary creatinine excretion noted in patients with renal insufficiency, which cannot be accounted for by changes in muscle mass or age [36]. In such circumstances, use of 1/Screat will overestimate ECC.

Interference with determination of creatinine. Most laboratory procedures for determining creatinine, including current auto-analyzer techniques, are based on the reaction of creatinine with alkaline picrate described more than a century ago [37]. However, this colorimetric reaction is not specific and many substances may interfere (Table 4). Normal serum always contains a certain amount of substances which react with alkaline picrate to produce a red colour. These so-called non-creatinine chromogens contribute 20% to total measured creatinine. In renal insufficiency the contribution of non-creatinine chromogens becomes less important [7]. Clinically important is the interference of aceto-acetate with creatinine determination. In patients with diabetes mellitus and ketoacidosis, and in patients after prolonged fasting, spurious increases of serum creatinine concentrations up to two-fold may be found [38–40]. In these instances, renal function is underestimated when using creatinine as marker of GFR. Many procedures and techniques have been developed which more or less circumvent these problems by measuring creatinine very specifically [41–43]. It is important to be aware of the possibility of interference and to check one's own laboratory method in this respect.

Other factors influencing serum creatinine and urinary creatinine excretion. In Table 2, several remaining factors are mentioned which may influence serum

creatinine and urinary creatinine excretion apart from a change in muscle mass or renal function. Creatinine production and excretion are increased after severe trauma, major surgery, and in cases of fever and sepsis [44]. A decreased creatinine excretion out of proportion to body weight is found in patients with excessive muscle wasting or severe muscular atrophy (immobilisation, neurological diseases). In the above-mentioned circumstances, serum creatinine will not reflect ECC, and may lead to a definite over- or underestimation of renal function.

Conclusion

Endogenous creatinine clearance is only a rough estimation of GFR. When precise information is needed, especially when studying small effects of therapeutic interventions, more invasive techniques should be used. However, in routine clinical practice a rough estimate of renal function will usually suffice. In these circumstances, determination of serum creatinine will almost always provide the required information, provided the possible pitfalls as mentioned in this review are taken into account.

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CHAPTER VI

PREDNISONE-INDUCED FLUCTUATIONS OF PROTEINURIA IN PATIENTS WITH A NEPHROTIC SYNDROME

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Prednisone-Induced Fluctuations of Proteinuria in Patients with a Nephrotic Syndrome

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Key Words. Prednisone · Nephrotic syndrome · Proteinuria · Renal hemodynamics

Abstract. We studied the effect of prednisone on urinary protein excretion in 19 patients with a nephrotic syndrome, who were treated with prednisone (125–150 mg) on alternate days. We found a typical, fluctuating pattern of proteinuria resulting from an increased protein excretion rate on prednisone days and a decreased protein excretion rate on nonprednisone days. The urinary protein excretion on prednisone days was 9.9 ± 3.3 g/24 h, as compared to 5.7 ± 3.8 g/24 h on nonprednisone days (mean ± SD). In the whole group of patients the percentual change in proteinuria was significantly correlated with the endogenous creatinine clearance. However, systematic differences between creatinine excretion rates on prednisone and nonprednisone days were not found in individual patients. In 6 patients, renal hemodynamics were studied more precisely, using a single injection technique. Only a slight and nonsignificant decrease in glomerular filtration rate was found on nonprednisone days (Δ = −9.6 ± 16.3%, mean ± SD). Filtration fraction remained unchanged. It is therefore suggested that the effects of prednisone on proteinuria are not simply mediated by overall changes in renal hemodynamics.

Glucocorticoid treatment is regularly used in patients with a nephrotic syndrome [1, 2]. Its therapeutic efficacy in patients with 'minimal change' disease is well established [3, 4]. A rapid decrease in urinary protein excretion rate can be observed in most of these patients. In 1979, beneficial effects of prednisone (P) treatment were reported in patients with membranous nephropathy [5]. The authors advised to use an alternate-day regimen, in order to reduce side effects.

There are a few reports in which mention is made of an increase of urinary protein excretion after administration of corticosteroids [6, 7]. However, after the initial observations in children of Heymann and Grupe [7], the phenomenon has not received further attention. While treating patients with a nephrotic syndrome according to the above mentioned Coggins' scheme [5], we observed an increase of proteinuria on P days [8]. We have tried to gain a better insight in this effect by carrying out systematic studies in more patients. As P causes changes in renal hemodynamics in experimental animals and in man [9, 10], which could be responsible for the effects observed, we have carried out additional, more precise measurements of glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). Our study shows that administration of P in an alternate day regimen results in a typical fluctuating pattern of proteinuria, due to an increased protein excretion rate on P days and a decreased protein excretion rate on nonprednisone (NP) days. The latter has not been described before and appears to be an independent phenomenon related to the withdrawal of corticosteroids. The results of the hemodynamic studies suggest that the observed fluctuations of protein excretion are not mediated by alterations in GFR or ERPF.

Materials and Methods

We studied 19 patients with a nephrotic syndrome, with a mean age of 42.1 years (range 15–72), and a mean endogenous creatinine clearance (ECC) of 109 ml/min (range 33–220). Clinical data are presented in table I. All but 3 patients had a membranous glomerulonephritis. Patient No. 17 was treated because of sarcoidosis. In patient No. 16 an erroneous diagnosis of membranous nephropathy was made initially. Patient No. 2 who was treated because of a

Table 1 Clinical data

Patient	Sex	Age	Diagnosis	ECC ml/min	Other medication
1	M	15	MG	220	
2	F	29	ML	175	-
3	M	37	MG	156	chlorthalidone propranolol cimetidine
4	M	33	MG	150	
5	M	34	MG	148	-
6	F	46	MG	134	
7	M	65	MG	125	-
8	F	16	MG	125	
9	M	48	MG	117	cimetidine
10	F	22	MG	117	
11	M	52	MG	115	cimetidine
12	M	54	MG	103	chlorthalidone
13	M	29	MG	100	furosemide cimetidine
14	F	36	MG	72	furosemide cimetidine
15	M	45	MG	62	cotrimoxazol
16	F	41	FSPG	45	cimetidine
17	M	66	FGS sarcoidosis	42	furosemide
18	M	61	MG	35	furosemide digoxin
19	F	72	MG	33	furosemide

M = male F = female MG = membranous glomerulonephritis ML = minimal lesions FSPG = focal segmental proliferative glomerulonephritis FGS = focal glomerulosclerosis ECC = endogenous creatinine clearance

minimal change disease is included because of the steroid resistant nature of her nephrotic syndrome

All patients were admitted to the hospital and treated with P in an alternate day regimen [5] Ten patients received 125 mg of P every other day the 9 other patients in whom body weight exceeded 80 kg received 150 mg of P Protein and creatinine excretion in 24 hour urine samples were measured on P and NP days In 3 patients the time course of proteinuria after intravenous prednisolone administration was studied by sampling urine 4 hourly during 6 consecutive days Comparison with pretreatment values of protein and creatinine excretion was possible in 10 patients who met with the following criteria (a) at least two 24 hour urine samples collected within the hospital immediately before starting P treatment and (b) no change in concomitant medication (patients in whom cimetidine treatment was started together with P treatment were thus excluded)

Creatinine concentrations in urine and serum were determined using Jaffe's method Urinary protein excretion was measured using the biuret method Percentual changes of proteinuria between P and NP days were calculated using the following formula

$$\frac{\text{Mean proteinuria P days} - \text{mean proteinuria NP days}}{(\text{Mean proteinuria P days} + \text{mean proteinuria NP days})\ 2} \times 100$$

A total of four 24 hour urine samples were considered to be collected incompletely as judged by a 40 50% decrease in creatinine excretion These samples were excluded from the calculations ECC were calculated from creatinine values measured in serum and in 24 hour urine samples

In 6 patients we measured GFR and ERPF using a single injection technique [11] ^{125}I iodothalamate and ^{131}I hippuran clearances were used as markers for GFR and ERPF respectively After rapid intravenous injection of the markers blood samples were drawn regularly during a 4 hour period In all cases the terminal monoexponential part of the curve was reached in some later than expected probably due to a decreased renal function and/or an increased volume of distribution due to the edema [12] In these patients analysis of the plasma disappearance curve according to an open two compartment model [13] could overestimate the renal clearance [14 15] We therefore analyzed the plasma disappearance curves according to both a two and a three compartment model except for two ERPF curves that could not be fitted well according to a three compartment model As expected renal clearances using the three compartment model were lower (GFR mean $\Delta = -10.9\%$ ERPF mean $\Delta = 11.8\%$) Both methods correlated well (GFR $r = 0.99$ ERPF $r = 0.98$) Therefore we chose the values obtained with the most commonly used two compartment model In all patients measurements were done on a P day and a NP day 5 days after starting therapy In 5 patients we did additional measurements immediately before starting therapy (control measurements) In 1 patient measurements were repeated 2 months after starting therapy All measurements were started at 13 00 h On P days P was administered 8 h before the start of the isotope study (at 5 00 h) All patients gave informed consent and they received KI 100 mg/day to protect the thyroid from radiation damage

For statistical analysis we used Wilcoxon's rank sum test and Student's t test when appropriate Linear regression was calculated using the Spearman correlation

Results

The time course of proteinuria in 3 patients after intravenous administration of prednisolone is shown in figure 1 A rapid increase of protein excretion was found reaching its maximum 8-12 h after administration of prednisolone No concomitant changes in creatinine excretion rates could be found Administration of P in an alternate day regimen resulted in a typical fluctuating pattern, as shown in figure 2 for all patients (mean number of observed P and NP days 12 4, range 6-20 days) The urinary protein excretion on P days was 9.9 ± 3.3 g/24 h (mean $\pm$ SD), as compared to 5.7 ± 3.8 g/24 h (mean $\pm$ SD) on NP days ($p < 0.001$) The fluctuations remained present during the whole observation period The figure could suggest that protein excretion decreased with time This however was not the case The apparent decrease of overall protein excretion was due to the fact that patients with the longest observation period had a

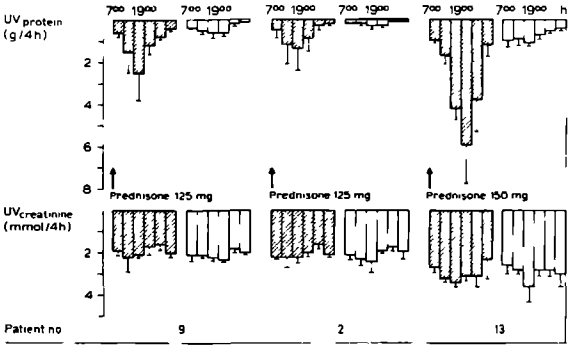

Fig. 1. Mean protein and creatinine excretion in 4-hour urine samples on P days (hatched bars) and NP days (open bars) in 3 patients. Urine samples were collected on 6 consecutive days. Values are given as means ± SD

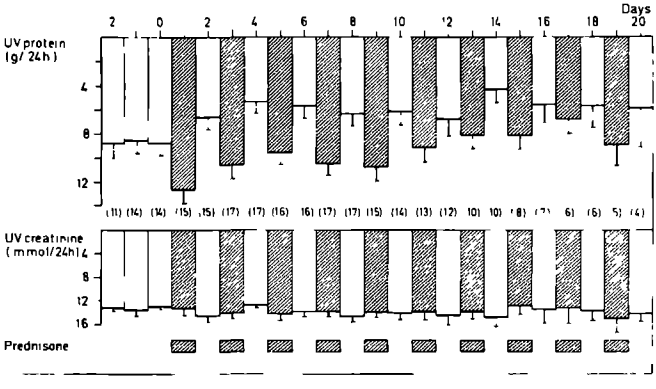

Fig. 2. Mean protein and creatinine excretion in 24 hour urine samples, before and during P therapy of all patients studied. P administration was started at day 1. Hatched bars represent P days, open bars represent NP days. Values are given as means ± SEM. The number of patients is given in parentheses

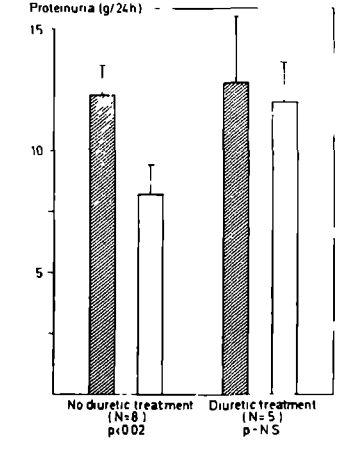

Fig. 3. Protein excretion rate on the first P day (hatched bars) and the consecutive P days (open bars) in patients without and with diuretic treatment. Values are given as means ± SEM

lower protein excretion rate. In individual patients no diminution of proteinuria on P or NP days was observed. In 2 patients (Nos. I and II) in whom we measured proteinuria when tapering the P dose, differences in protein excretion rates could still be observed with a dose as low as 15 mg every other day.

Figure 2 shows that proteinuria on the first P day exceeded that on the following P days. On further analysis, this difference appeared to be significant only in patients not using diuretics (fig. 3). Patients without diuretics ($n=8$) had a mean ECC of 145 ± 37 ml/min, whereas in patients with diuretics ($n=5$) the ECC was 85 ± 52 ml/min (mean ± SD; $p=0.07$). Within each group there was no correlation of ECC with the difference in proteinuria between the first and subsequent P days.

In 10 patients the protein excretion rate on P and NP days could be compared with control protein excretion rates measured immediately before the start of the treatment (fig. 4). The increase of protein excretion on P days

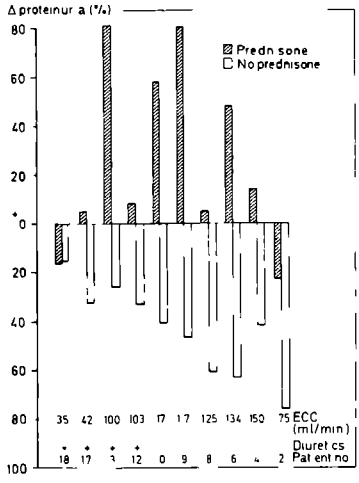

Fig 4 Percentual change in protein excretion rate on P days (hatched bars) and NP days (open bars) as compared to pretreatment values Patients are arranged according to ECC

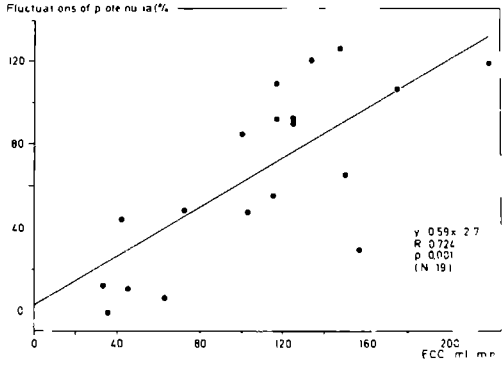

Fig 5 Relationship between percentual fluctuations of protein uria and the ECC

(Δ = +25 9 ± 38 3%, mean ± SD) was less consistent than the decrease of protein excretion on NP days (Δ = 43 9 ± 18 6%, mean ± SD) In patients with a high ECC (ECC ⩾ 125 ml/min), the increase in proteinuria on P days seems less pronounced The decrease of protein excretion on NP days was not correlated with the increase of protein excretion on P days, neither in the whole group, nor in the subgroup of patients with normal or

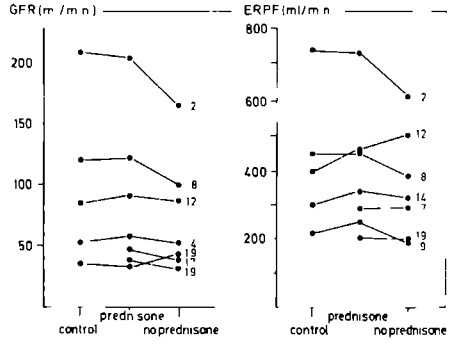

Fig 6 Results of GFR and ERPF measurements in 6 patients The patient number is given at the right of each curve Patient 19 was measured twice

high ECC (⩾ 100 ml/min) It should be realized that patient No 2 with 'minimal change disease is included in this study Although this patient did not reach a remission during treatment, it is possible that a partial therapeutic effect of P was responsible for the decrease of proteinuria on P days

The percentual change of protein excretion between P and NP days in individual patients was significantly correlated with the ECC (fig 5) A similar correlation with ECC was found for the decrease of protein excretion on NP days as compared to pretreatment values However, urinary creatinine excretion rates on P and NP days were not different (P days 13 6 ± 3 2 mmol/24 h, NP days 13 8 + 3 0 mmol/24 h, mean ± SD) Results of GFR and ERPF measurements are shown in figure 6 Differences are small, GFR on NP days tending to be lower than on P days (Δ = −9 6 ± 16 3%, mean ± SD p = 0 10) No significant changes in filtration fraction (i e GFR/ERPF) were observed

Discussion

Our study partly confirms earlier observations of a corticosteroidinduced increase in proteinuria [6, 7] In Heymann and Grup s study [7] a maximal effect was found on the first day, in agreement with our observations in patients not using diuretics Heymann and Grupe [7] found that during daily administration of P, the proteinuric effect wore off after a few days Using an alternate-day regimen of P administration we observed no diminution in P-induced fluctuations of proteinuria dur-

ing a treatment period of 2 months A trivial explanation for the observed decrease of protein excretion rate on NP days might be that the increased protein excretion on P days had lowered serum protein concentration Assuming that GFR remained unaltered, this could cause a slight decrease in protein excretion on NP days However, in 4 patients the observed fluctuations in proteinuria were caused by a mere decrease of protein excretion on NP days, not preceded by an increase on P days Moreover, the increase of protein excretion on P days was not correlated with the decrease on NP days From this we conclude that the decreased protein excretion rate on NP days is an independent phenomenon related to the withdrawal of P

Although an untreated control group is lacking in our study, it seems unlikely that the fluctuations in protein excretion occurred spontaneously Data on 24-hour protein excretion in hospitalized patients are scarce In an earlier study only slight variations were found [16], as we did in 5 patients who were hospitalized from 5 to 7 days before starting P therapy In patients studied under standard conditions with strict bed rest, a gradual decrease in 24 hour protein excretion on consecutive days is found [17] In these patients a circadian rhythm was observed with fluctuations of protein excretion within a 24 hour period However, the fluctuations that we observed would require a 48-hour rhythm in protein excretion Such a rhythm was not noted in the last mentioned study We therefore feel that the fluctuations we observed are the result of alternate day P administration

In the rat a glucocorticoid induced increase in protein excretion is also found [18], and this has been shown to be dose dependent [19] Time relations differ from our data however, the increase reaching its maximum not until 14–32 h after glucocorticoid administration In the 3 patients in whom we studied this, the proteinuric effect was already apparent after 4 h and reached a maximum after 8–12 h We did not systematically study dose-effect relations, but we still noted fluctuations in protein excretion rates in patients receiving P doses as low as 15 mg

Several factors could be responsible for the observed changes in protein excretion rate alterations in renal hemodynamics, changes in tubular reabsorption of protein, or changes in the permselective characteristics of the glomerular basement membrane [20] The correlation we found between the percentual change in protein excretion and the ECC could be explained by an effect of P on renal hemodynamics assuming hyperfiltration in remnant functioning nephrons [21] This hyperfiltration decreases the functional reserve capacity of the kidney [22] Consequently the ability of the kidney to increase GFR and renal blood flow will be lost in patients with renal insufficiency However, a tubular mechanism cannot be excluded In renal insufficiency there will be an absolute loss of functioning nephrons, and therefore of tubuli If compensatory hyperfiltration occurs, ECC will tend to overestimate the amount of functioning tubuli Consequently, tubular effects of P will be less prominent in patients with renal insufficiency and therefore a similar correlation of the percentual change of protein excretion and the ECC can be expected

It is difficult to derive from the data reported in the literature whether the proteinuric effect of P is primarily glomerular or tubular Chronic administration of glucocorticoids increases GFR in animals and man [9, 10, 23 25] Observations on the acute effects of glucocorticoid administration are less straightforward In the rat an increase of GFR is found [18], but in man GFR was found to remain unchanged [26], increased [27], or even decreased [28] Glucocorticoids decrease proximal tubular reabsorption of water and electrolytes, and probably amino acids in man [29–31] In the rat an effect of prednisolone on tubular protein reabsorption could not be found [18]

We did not find fluctuations in creatinine excretion rates indicating unaltered GFR on P and NP days However, creatinine clearance is an unreliable marker of GFR especially in patients with a nephrotic syndrome [32] Furthermore, P could inhibit tubular secretion of creatinine thus masking any positive effect on glomerular filtration of creatinine [28] Therefore, we studied renal function more precisely, using a single injection technique Only minor fluctuations in GFR and ERPF were found It is therefore unlikely that the fluctuations in protein excretion rates can be explained by changes in GFR This is consistent with the findings in the rat, where the increase in protein excretion was not accompanied by an increase in GFR [18] Since we did not measure GFR on the first P day, we cannot exclude an acute hemodynamic effect of P which would explain the extra increase in protein excretion rate on the first P day observed in patients not using diuretics Attenuation of this first day effect by diuretics suggests alterations in renal hemodynamics, since diuretics are known to influence ERPF and renal autoregulation [33, 34] This phenomenon and also the effects of P administration on tubular function deserve further study

Our observations of a consistent decrease in protein excretion rate on NP days points to another possible mechanism Renin, angiotensin as well as norepineph

rine are known to induce proteinuria [35–37] Since these effects are blocked by adrenalectomy it is assumed that glucocorticoids have a permissive action on generating this proteinuria This permissive action of glucocorticoids cannot be explained by mere changes in renal hemodynamics [38] Assuming such a permissive action of glucocorticoids a decrease of endogenous cortisol on NP days could have been responsible for the observed decrease in protein excretion rate By a similar mechanism the circadian rhythm of endogenous cortisol production might be responsible for the observed 24 hour fluctuations of proteinuria in untreated patients with the nephrotic syndrome [17]

We conclude that P administered in an alternate day regimen causes changes in protein excretion rates which are not explained by hemodynamic alterations Further studies are necessary to elucidate the responsible mechanisms From a practical point of view it is important to realize that transient increases or decreases of proteinuria during treatment with P do not necessarily reflect therapeutic failure or success especially when intermittent dosage schedules are used

Acknowledgements

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CHAPTER VII

PREDNISOLONE CAN INCREASE GLOMERULAR PERMEABILITY TO PROTEINS IN NEPHROTIC SYNDROME

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Prednisolone can increase glomerular permeability to proteins in nephrotic syndrome

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Prednisolone can increase glomerular permeability to proteins in patients with a nephrotic syndrome In patients with a nephrotic syndrome administration of prednisolone causes an increase of proteinuria To elucidate the mechanism of this effect we have studied the acute proteinuric effect of prednisolone 125 to 150 mg intravenously in nine patients (7 M 2 F) with a nephrotic syndrome Mean age (± SD) of the patients was 53 ± 6 years mean endogenous creatinine clearance 104 ± 30 ml/min and mean proteinuria 7 7 ± 3 0 g/24 hr After administration of prednisolone urinary total protein excretion rose in all patients from a mean (±SEM) of 4 89 ± 0 59 mg/min before to 9 09 ± 0 99 mg min at five hours after administration ($P < 0.01$) Glomerular filtration rate (inulin clearance) effective renal plasma flow (PAH clearance) and filtration fraction did not change significantly The increases of urinary excretion of albumin (median % +92%) IgG (median % +88%) and transferrin (median % +76%) were comparable and correlated significantly Urinary excretion of β2 microglobulin did not change significantly however We conclude that intravenous administration of prednisolone to patients with a nephrotic syndrome causes an increase in urinary protein excretion rate which cannot be explained by changes in renal hemodynamics or tubular protein reabsorption and which therefore must be the result of a change in glomerular permselectivity characteristics

Glucocorticoid treatment is regularly used in patients with a nephrotic syndrome due to minimal change disease or membranous nephropathy [1 2] In order to reduce side effects of corticosteroid therapy an alternate day regimen has been advised [2] While treating patients with a nephrotic syndrome according to the above mentioned Coggin s scheme [2] we observed a typical fluctuating pattern of proteinuria due to an increased proteinuria on prednisone days and a decrease on non prednisone days [3 4] The increase of proteinuria seemed most prominent on the first day of prednisone treatment [4] Similar observations of a corticosteroid induced increase of proteinuria have been made before in human [5 6] as well as in animal studies [7 8] The mechanism of this effect is as yet unclear Therefore we have studied the acute effects of prednisolone on protein excretion rate in patients with a nephrotic syndrome and concurrently measured changes in renal hemodynamics and tubular protein reabsorption Our study demonstrates that prednisolone acutely increases proteinuria without causing changes in renal hemodynamics or tubular protein reabsorption This suggests that prednisolone increases proteinuria by causing a change in glomerular permselectivity

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Methods

We studied nine patients with a nephrotic syndrome due to idiopathic membranous glomerulonephritis (N = 7) or minimal change disease (N = 2) Mean age (± SD) of the patients was 53 ± 6 years mean endogenous creatinine clearance (ECC) was 104 30 ml/min and mean blood pressure 141 89 ± 14 8 mm Hg No patient had evidence of orthostatic hypotension Further clinical data of the patients are presented in Table 1

All patients were studied on the day of the start of prednisolone therapy All but two patients were admitted to the hospital several days before All patients were on a normal sodium intake diet containing 100 to 150 mmol Na After a light breakfast patients came to the investigating room at 9 00 a m where the experiment took place from 9 00 a m till 5 00 p m No food was given during the experiment A firm diuresis was established by an initial oral water load of 500 to 1000 ml Thereafter we tried to maintain urinary flow rate above three ml min by giving water orally or infusing a 5% glucose solution To keep urinary pH above 6 0 (which is necessary for correct measurement of β2 microglobulin vide infra) one gram of sodium bicarbonate was given at regular intervals of 60 to 120 minutes Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured using a continuous infusion technique Renal clearances of inulin (Inutest® Laevosan GMBH Linz Austria) and para aminohippurate (PAH) were used as markers of GFR and ERPF respectively After an equilibration period of 90 minutes urine was collected at regular intervals (45 to 60 min) for determination of inulin PAH sodium total protein albumin transferrin IgG and β2 microglobulin (β2M) In seven patients urine was collected using a bladder catheter in two patients it was collected by spontaneous voiding After collecting two 45 minute urine samples for obtaining base line values prednisolone sodium succinate was administered intravenously in a dose of 125 mg (body weight ≤ 75 kg) or 150 mg (body weight > 75 kg) After administration of prednisolone a total of five urine samples were collected at intervals of 60 minutes Before and at the end of every urine collection period blood for determination of inulin PAH and sodium was sampled via a second indwelling venous catheter Furthermore in seven patients blood samples were

Table 1 Clinical data of the patients

Patient	Sex	Age years	Diagnosis	ECC ml/min	Proteinuria g/24 hr	Other medication
1	M	53	MGN	114	8 3	—
2	M	44	MGN	85	6 9	pindolol/ clopamide
3	M	57	MGN	86	4 4	atenolol
4	M	56	MGN	113	6 3	—
5	M	58	MGN	155	6 9	metoprolol
6	F	49	MI	128	5 8	—
7	M	54	ML	118	12 9	aceno coumarol
8	M	55	MGN	85	5 6	—
9	F	50	MGN	51	12 4	metoprolol

Abbreviations are M male F female MGN membranous glomerulonephritis ML minimal lesions ECC endogenous creatinine clearance (determined from serum creatinine and 24-hr urinary creatinine excretion)

collected immediately before, and three and five hours after administration of prednisolone for determination of albumin, transferrin, IgG and β_2M

Sodium, inulin, and PAH in urine and plasma were measured using standard semi-automated techniques Urinary total protein was measured using the biuret method Albumin, transferrin, and IgG in urine and serum were measured by immunonephelometry using specific antibodies raised in rabbits Serum and urinary β_2M were measured using a commercially available radioimmunoassay (Pharmacia) In one patient no serum PAH and sodium values could be determined due to technical difficulties In another patient urinary β_2M was not measured because of a low urinary pH (pH < 6 0) Filtration fraction (FF) was defined as GFR/ERPF The fractional excretion (FE) of a substance was defined as the renal clearance of that substance divided by the GFR, and expressed as a percentage For statistical analysis Wilcoxon's sign rank test was used Linear regression was calculated using Spearman's correlation coefficient A P value less than 0 05 was considered significant Unless otherwise stated all values are given as means ± SEM

The study protocol was approved by the Hospital Ethical Committee All patients gave informed consent

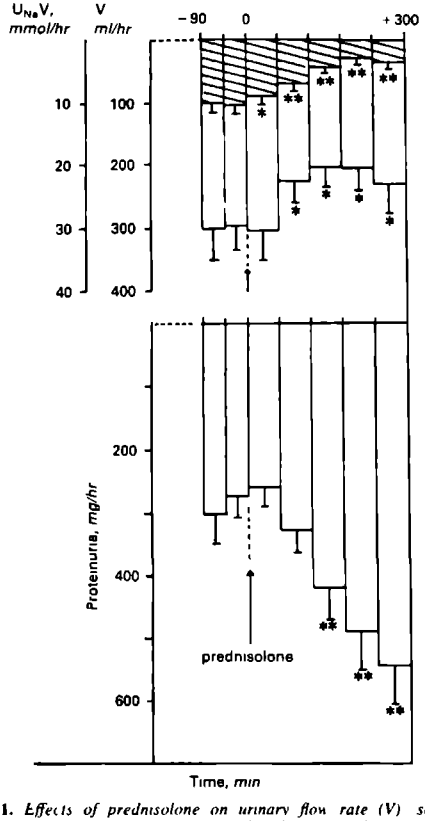

Fig. 1. *Effects of prednisolone on urinary flow rate (V) sodium excretion ($U_{Na}V$) and proteinuria* Prednisolone was administrated at time = 0 min Baseline values were obtained from −90 to 0 min *P < 0 05 **P < 0 01 compared to mean baseline values

Results

The hydration protocol resulted in a diuresis of more than 3 ml/min in all but one patient After administration of prednisolone urinary flow rate decreased slightly but significantly from 5 0 ± 0 6 ml/min to a lowest mean value of 3 3 + 0 5 ml/min (299 ± 38 ml/hr and 197 ± 32 ml/hr, respectively, Fig 1) Urinary sodium excretion was also significantly reduced (Fig 1) FL_{Na} decreased from 1 96 ± 0 42% to a lowest mean of 0 63 ± 0 21% (N = 8, P < 0 02) Serum albumin increased significantly from 23 9 ± 1 3 g/liter to 25 9 ± 2 0 g/liter (P < 0 05) Similar increases were found for IgG and transferrin (IgG 4 81 ± 0 72 g/liter to 5 13 ± 0 80 g/liter, P < 0 05 Transferrin 1 81 ± 0 22 g/liter to 1 98 ± 0 14 g/liter, P < 0 05)

After administration of prednisolone urinary total protein excretion increased significantly, from a mean value of 4 89 ± 0 59 mg/min before to 9 09 ± 0 99 mg/min at five hours after administration of the drug (293 ± 35 mg/hr and 545 ± 59 mg/hr, respectively Fig 1) As shown in the figure an increase of urinary protein excretion was already apparent at two hours after administration of prednisolone Urinary total protein excretion increased in all patients, percentual increases (after five hours) ranging from +21% to +178% (median −95%)

The renal hemodynamic effects of prednisolone are shown in Figure 2 Although two patients demonstrated a clear increase of ERPF at four to five hours after administration of prednisolone no significant changes in GFR or ERPF occurred overall As a result filtration fraction remained virtually unchanged during the experiment

Overall effects of prednisolone on the urinary excretion of the various proteins measured are given in Table 2, showing parallel and significant increases of urinary albumin, IgG, and transferrin excretion but no significant change in urinary β_2M excretion The excretion rates for the individual patients of urinary albumin, IgG, transferrin, and β_2M at the start and during the last hour of the experiment are shown in Figure 3 It is clear from this figure that absolute urinary excretion rates of

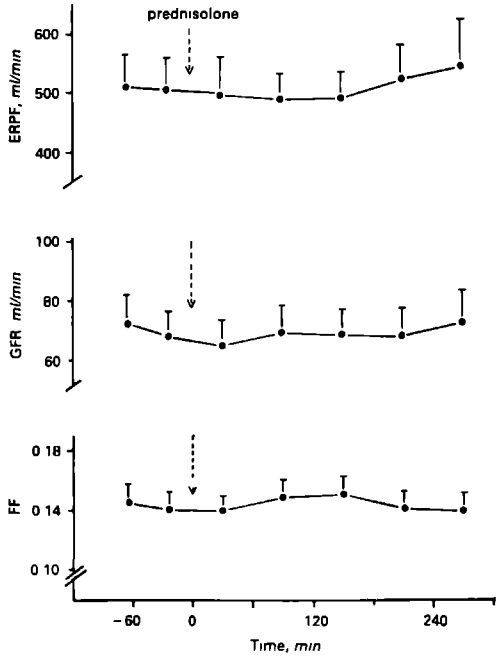

Fig. 2 *Acute effects of prednisolone on renal hemodynamics* Abbreviations are GFR glomerular filtration rate ERPF effective renal plasma flow FF filtration fraction

albumin, IgG and transferrin rose in all patients Fractional excretion of albumin IgG and transferrin (which could be calculated in seven patients) also rose in all patients (albumin from 0 374 ± 0 098% to 0 578 ± 0 095% $P < 0\ 05$ IgG from 0 066 ± 0 034% to 0 117 ± 0 036% $P < 0\ 05$ transferrin from 0 316 + 0 095% to 0 561 ± 0 110%, $P < 0\ 05$) Percentage increases of urinary albumin IgG and transferrin excretion were comparable (albumin median Δ% +92% IgG median Δ% +88%, transferrin median Δ% +76%) and correlated significantly (albumin vs transferrin $r = 0\ 70$ $P < 0\ 05$, albumin vs IgG $r = 0\ 68$, $P < 0\ 05$ and transferrin vs IgG $r = 0\ 80$ $P < 0\ 01$, Fig 4) From Figure 4 it is apparent that the increase of urinary IgG excretion exceeded the increase of urinary transferrin excretion in seven out of nine patients However, this difference was not significant ($P = 0\ 08$) As a result selectivity of proteinuria (calculated as clearance IgG/clearance transferrin) did not change significantly Although overall urinary excretion of β_2M did not change significantly (Table 1) an increase was found in six patients (Fig 3) The percentual increase of urinary β_2M excretion correlated with the percentual increase of urinary IgG excretion ($r = 0\ 76$ $P < 0\ 05$) but was always less The percentual increase of urinary β_2M excretion was less than the percentual increase of albumin excretion in seven patients (out of eight) Consequently the ratio of urinary β_2M excretion/urinary albumin excretion decreased significantly from 0 475 ± 0 304 to 0 308 ± 0 174 ($P < 0\ 05$)

Discussion

We recently have confirmed earlier observations in humans of a corticosteroid induced increase of proteinuria [3, 4] To elucidate the mechanism of this effect we have studied the acute effects of prednisolone in nine patients with renal disease and proteinuria

All patients were on a normal sodium intake when studied We noted a slight decrease of urinary flow rate and a firm decrease of urinary sodium excretion after administration of prednisolone Although high doses of prednisolone have been shown to exert mineralocorticoid effects [9] which could explain the antinatriuresis that we observed the decreased urinary flow rate could also be the result of incomplete replacement of urinary losses This is underscored by the slight increases of serum albumin transferrin and IgG that we noted These small increases of serum protein concentrations cannot explain the observed changes in proteinuria in view of the marked increases in the fractional excretion of albumin IgG and transferrin Our study therefore convincingly demonstrates that prednisolone acutely increases proteinuria in patients with a nephrotic syndrome In the rat a glucocorticoid induced increase of protein excretion is also found [7 8] Time relations differ however since we noted an increased protein excretion rate as soon as three hours after administration of prednisolone, whereas in rats an increased protein excretion rate is not found before eight hours after administration of the drug

Several factors could be responsible for the observed rise in protein excretion rate alterations in renal hemodynamics changes in tubular protein reabsorption or changes in the permselective characteristics of the glomerular basement membrane [10] Chronic administration of corticosteroids increases GFR in animals and man [11–15] In man this is most likely the result of corticosteroid induced increases of plasma and extracellular volume [13] Observations on the acute renal hemodynamic effects of corticosteroid administration in man are less straightforward Although most authors agree that in human individuals with normal adrenal function no consistent changes in GFR or ERPF occur within the first hours after administration of corticosteroids [16–19] occasionally increases as well as decreases of both GFR and ERPF have been noted [20 21] In our patients the increases of protein excretion were not accompanied by significant changes in GFR and ERPF Furthermore, in the few patients who showed an increase of ERPF or GFR at the end of the experiment these changes were always preceded by the increases of proteinuria Taken together this makes a major role for renal hemodynamics in the proteinuric effect of prednisolone less likely However since in human studies only information can be obtained on overall renal function an effect through redistribution of renal blood flow cannot be completely excluded

Corticosteroids interfere with proximal tubular transport of water and electrolytes as well as of amino acids [15 19, 22, 23] We have used urinary excretion of β_2-microglobulin an anionic low molecular weight protein (MW 11 600) as a marker of tubular protein reabsorption [24 25] Under normal circumstances up to 99% of the filtered β_2M is reabsorbed by the proximal tubules Tubular reabsorption of albumin is less

Table 2. Prednisolone induced changes in urinary excretion of total protein albumin transferrin IgG and β_2-microglobulin

Time *min*	Urinary excretion *mg/min*				
	Total protein	Albumin	Transferrin	IgG	β_2M × 10^3
−90 to −45	5 18 ± 0 65	4 58 ± 0 53	0 34 ± 0 06	0 13 ± 0 03	1 94 ± 1 21
−45 to 0	4 60 ± 0 54	4 20 ± 0 46	0 31 ± 0 06	0 12 ± 0 03	1 92 ± 1 22
0 to 60	4 40 ± 0 48	4 00 ± 0 37	0 30 ± 0 06	0 11 ± 0 03	1 53 ± 0 95
60 to 120	5 55 ± 0 51	4 68 ± 0 52	0 36 ± 0 07	0 15 ± 0 04	1 68 ± 1 00
120 to 180	7 00 ± 0 80[b]	6 08 ± 0 63[b]	0 50 ± 0 09[b]	0 23 ± 0 07[b]	2 10 ± 1 36
180 to 240	8 21 ± 0 99[b]	7 36 ± 0 92[b]	0 58 ± 0 10[b]	0 31 ± 0 12[a]	2 08 ± 1 25
240 to 300	9 09 ± 0 99[b]	8 21 ± 0 88[b]	0 65 ± 0 11[b]	0 29 ± 0 07[b]	1 77 ± 0 81

All values are given as mean ± SEM Prednisolone was administrated at time − 0 min Baseline values were obtained from −90 min till 0 min [a] $P < 0\ 05$ and [b] $P < 0\ 01$ compared with mean baseline values

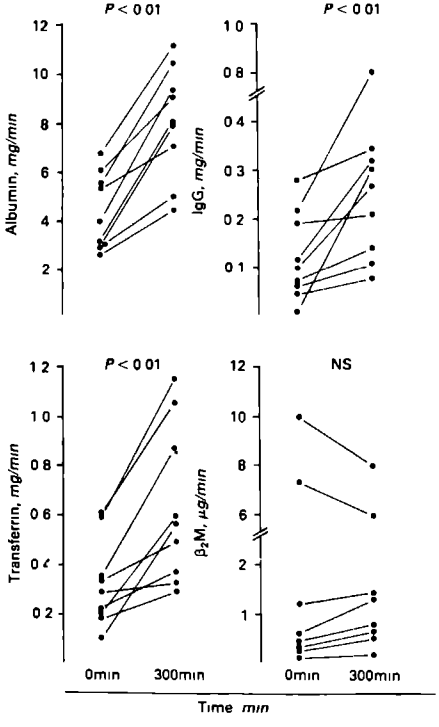

Fig. 3. *Acute effects of prednisolone on urinary excretion rates of albumin, transferrin, IgG and $\beta_2$microglobulin (β_2M)* Values obtained at the start (0 min) and in the last hour (300 min) of the experiment are shown for the individual patients

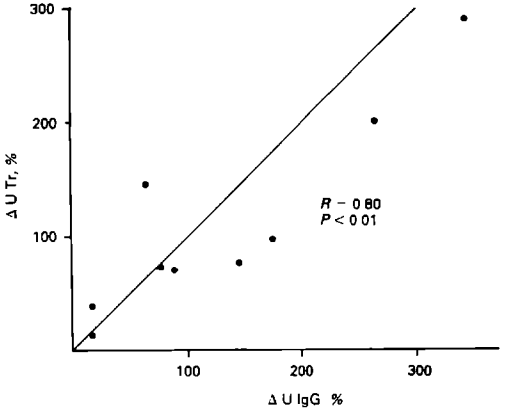

Fig. 4. *Correlation of the increase of urinary transferrin excretion (Δ UTr) with the increase of urinary IgG excretion (Δ UIgG)* For comparison the line of identity is shown

efficient After blockade of tubular protein reabsorption the relative increase of urinary β_2M excretion exceeds the relative increase of urinary albumin excretion severalfold [26] This also explains the increased ratio of urinary β_2M/urinary albumin found in patients with tubular interstitial disease [24] In our patients we observed no change in mean urinary β_2M excretion Individual responses varied, a decrease being found in two patients, an increase in the other six patients Percentual increase of urinary β_2M excretion was always less then the increase of urinary IgG excretion, and in all but one patient less then the increase of urinary albumin excretion This is reflected by a significant decrease of the ratio urinary β_2M excretion/ urinary albumin excretion, which, as indicated above, argues against a blockade of tubular protein reabsorption Furthermore, in the case of a defect in tubular protein transport proteinuria hardly ever exceeds 2 g/24 hr, equalling 1 5 to 2 mg/min [27] In most of our patients the increase in protein excretion rate was considerably greater, which provides further evidence that the proteinuric effects of prednisolone are not mediated by a decrease in tubular protein reabsorption

With the necessary precautions in mind we conclude that the proteinuric effects of prednisolone cannot be explained by changes in renal hemodynamics or tubular protein reabsorption, and thus must result from a change in glomerular permselectivity characteristics In rat studies the same conclusions were reached [7, 8] Although corticosteroids influence GFR in rats, the increase in proteinuria occurring 8 to 34 hours after

administration of the drugs was not accompanied by increases in GFR or lysozyme excretion

In recent years much has been learned about the permeability characteristics of the glomerular capillary wall [28] It has become evident that the transport of macromolecules through the glomerular filter is determined not only by molecular size and configuration (size-selectivity) but also to an important degree by molecular charge (charge selectivity) The negatively charged glomerular basement membrane impairs filtration of anionic proteins (such as albumin) Selective loss of glomerular basement membrane charges would result in a preferential loss of albumin We have studied the urinary excretion of proteins with different charge and molecular size such as albumin transferrin, and IgG The increases of urinary excretion of these proteins were comparable, thus favoring a predominant change in glomerular size-selectivity

It is difficult to derive from data reported in the literature how corticosteroids could change glomerular permselectivity Since adrenalectomy impairs the proteinuric effects of renin and norepinephrine in rats, it is assumed that corticosteroids have a permissive action on generating this proteinuria [29 30] Corticosteroids increase plasma renin activity [31] In the light of these observations it is tempting to speculate that changes in the renin angiotensin system contribute to the proteinuric effects that we have observed Since we did not measure plasma renin activity or angiotensin II in our patients we cannot determine their influence on the proteinuric effect of prednisolone However, in view of the homogeneous response in our patient group it would have been difficult to relate changes in proteinuria to basal levels of renin or angiotensin II Furthermore, angiotensin II increases proteinuria mainly by causing vasoconstriction of the efferent arteriole, leading to an increased glomerular capillary pressure which is reflected in an increased filtration fraction [29] Recently it was suggested that the proteinuria could be the result of binding of the cationic angiotensin II to the GBM and the subsequent decrease of GBM negative charge [30] The complete absence of changes in FF in our study and the absence of a predominant increase of albumin excretion indicate that the nature of the proteinuria after administration of prednisolone is quite different making a contribution of the renin-angiotensin system less likely

In our study population the response to prednisolone was quite homogeneous an increased proteinuria occurring in all patients However, we only studied patients with minimal change disease or membranous glomerulonephritis, and nephrotic range proteinuria Therefore one should be careful to apply conclusions drawn from our study to patients with renal disease and proteinuria from other causes In fact, from an ongoing study we have indications that patients with a proliferative glomerulonephritis do not respond to corticosteroids in a similar way Up to this moment the observed effects do not seem to have any relation with therapeutic outcome However, it is important to realize that during alternate day prednisone therapy transient changes in proteinuria can occur, which do not reflect therapeutic failure or success

In conclusion prednisolone can acutely increase proteinuria in patients with nephrotic syndrome The absence of changes in renal hemodynamics and renal tubular protein reabsorption suggest that this effect is caused by changes in glomerular permselectivity How the changes in glomerular permselectivity are effectuated remains to be determined

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CHAPTER VIII

DECREASES OF PROTEINURIA DURING ALTERNATE-DAY PREDNISONE THERAPY IN PATIENTS WITH MEMBRANOUS GLOMERULONEPHRITIS

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Submitted

ABSTRACT.

Treatment of patients with membranous glomerulonephritis [MG] with high-dose prednisone on alternate days results in a decreased protein excretion rate on non-prednisone days, which is not related to any therapeutic effect of prednisone. We have studied this phenomenon in more detail in 14 patients [11 M, 3F] with MG. Mean age [±SD] of the patients was 47±14 years, mean endogenous creatinine clearance 94±35 ml/min, and median proteinuria 8.8 g/24h [range 5.0-30.0 g/24h]. Glomerular filtration rate [GFR, inulin clearance], effective renal plasma flow [ERPF, PAH clearance], and proteinuria were measured on a control day [C], and six days after start of alternate-day prednisone treatment, on the third non-prednisone day [NP3, 24-28 hours after the last dose of prednisone]. Proteinuria decreased from 6.1 mg/min [3.2-9.8 mg/min] [C] to 2.5 mg/min [1.0-7.7 mg/min] at NP3 [median, interquartile range; $p<0.01$] The percentual decrease averaged 45±8%. The decrease of proteinuria was correlated with baseline GFR [$r=0.75$;$p<0.01$]. GFR and ERPF did not change significantly, but filtration fraction decreased significantly from 14.5 ± 0.8% [C] to 13.5 ± 0.9% [NP3; $p<0.05$]. In 12 patients we measured urinary excretion rates of albumin, IgG, transferrin, and β2-microglobulin. Urinary excretion of all these proteins decreased significantly, by -39 ± 9%, -60 ± 5%, -50 ± 9%, and -24 ± 10% respectively. The selectivity index of the proteinuria was not different on the study days. We conclude that proteinuria is decreased on NP days in patients with MG treated with alternate-day steroids. In view of the known permissive effect of corticosteroids on proteinuria, this decrease of proteinuria might be related to a depressed endogenous cortisol production on NP3 [serum cortisol C: 0.27 ± 0.04 mmol/l, NP3: 0.10 ± 0.02 mmol/l; $p<0.01$]. Mechanisms which are probably involved in this decrease of proteinuria are a decrease of intraglomerular capillary pressure, and/or a change in glomerular permselectivity characteristics.

INTRODUCTION.

Glucocorticoid treatment is regularly used in patients with a nephrotic syndrome caused by membranous glomerulonephritis [1]. While treating these patients with high dose prednisone on alternate days, we observed a typical fluctuating pattern of proteinuria, due to an increased proteinuria on prednisone-days and a decrease on non-prednisone days [2,3]. We subsequently demonstrated that intravenously administered prednisolone increased proteinuria in these patients, thus confirming earlier observations of a corticosteroid-induced increase of proteinuria [4,5]. The increased proteinuria could not be explained by changes in renal hemodynamics or tubular protein reabsorption, suggesting that prednisolone increases glomerular permeability to proteins [4]. A recent study from Japan confirmed these observations [6]. By using fractional clearances of dextran these investigators demonstrated differences in glomerular permeability on prednisone and non-prednisone days. Although the observed fluctuations in proteinuria during alternate day prednisone therapy may solely be the result of an increased proteinuria on prednisone days, it cannot be excluded that in addition proteinuria becomes reduced on the non-prednisone day. We have prospectively studied this possibility in detail in patients with membranous glomerulonephritis, who were treated with high-dose alternate-day prednisone. The results demonstrate that proteinuria is decreased on non-prednisone days. This decrease of proteinuria might be related to a decreased endogenous cortisol production on non-prednisone days, which would be compatible with the known permissive effects of corticosteroids on proteinuria in animals [7].

PATIENTS AND METHODS.

We studied 14 patients with a nephrotic syndrome caused by biopsy-proven membranous glomerulonephritis. Mean age [± SD] of the patients was 47 [± 14] years, mean endogenous creatinine clearance [ECC], as calculated from serum creatinine and 24h

urinary creatinine excretion, 94 ± 35 ml/min. Three patients were treated for hypertension. Pertinent data of the individual patients are given in Table I. In all but one patient membranous glomerulonephritis was considered idiopathic. In one patient [no.3] the nephrotic syndrome was related to penicillamine treatment for active rheumatoid arthritis.

All but one patient were admitted to the hospital before start of prednisone treatment. For the whole group of patients sodium intake ranged from 50 to 200 mmol/day, but it was kept constant in the individual patient. A substantial part of this sodium was supplied by sodium bicarbonate which was given in a dose of 3-6 g/day, in order to keep urinary pH above 6.0 [which was necessary for correct measurement of β2-microglobulin; vide infra]. Patients were treated with prednisone orally in a dose of 125 mg [body weight <80 kg] or 150 mg [body weight >80 kg] on alternate days.

Detailed studies of renal function and proteinuria were done on a control day [C, day before start of prednisone treatment] and

Table I. Patient characteristics.

Patients No.	Sex	Age (years)	Proteinuria (g/24h)	ECC (ml/min)	Therapy
1	F	47	11.6	74	Fu,C
2	M	37	9.8	90	Fu
3	M	70	8.9	97	H,I
4	F	38	5.0	150	----
5	M	54	5.0	81	----
6	M	58	5.5	90	M,D,Dil
7	M	59	5.8	113	----
8	F	52	12.4	51	M,E
9	M	41	30.0	69	Fu
10	M	27	6.2	86	Fu
11	M	40	8.7	141	Fu
12	M	68	12.0	23	Fu
13	M	24	5.2	121	----
14	M	42	15.9	134	A

Abbreviations: ECC=endogenous creatinine clearance. Fu=furosemide,C=captopril,I=indomethacin,H=hydroxychloroquine,M=metoprolol,D=dipyridamol,Dil=diltiazem,E=endralazine, A=atenolol.

six days after start of treatment, i.e. on the third non-prednisone day [NP3]. All studies were done between 9.00 a.m. and 1.00 p.m., to exclude any influence from diurnal rhythms. The patients took a light breakfast on the study day. A sufficient diuresis was established by an oral water load of 500-1000 ml. Thereafter, we tried to maintain diuresis by giving water orally. Glomerular filtration rate [GFR] and effective renal plasma flow [ERPF] were measured using a continuous infusion technique. Renal clearances of inulin [Inutest^{R} - Laevosan GMBH, Linz, Austria] and para-amino-hippurate [PAH] were used as markers of GFR and ERPF, respectively. After an equilibration period of 90 min, two or three urine samples were collected at regular intervals [30-60 min] for determination of creatinine, inulin, PAH, sodium, total protein, albumin, transferrin, IgG and β2-microglobulin [β2M]. Urine was collected by spontaneous voiding. At the mid-point of the urine collection periods, blood was drawn for determinations of inulin, PAH, creatinine, sodium, albumin, IgG, transferrin, β2M, total protein, and hematocrit. In addition blood samples were drawn at the start of the experiments to determine inulin and PAH "blanks". At this time a blood sample was drawn for determination of cortisol.

Sodium, creatinine, inulin and PAH in urine and serum were measured using standard semi-automated techniques. Urinary total protein was measured using the biuret method. Plasma cortisol was measured using a specific radioimmunoassay. Albumin, transferrin and IgG in urine and serum were measured by immunonephelometry using specific antibodies raised in rabbits. Serum and urinary β2M were measured using a commercially available radioimmunoassay [Pharmacia]. In one patient, no serum PAH values could be determined due to technical difficulties. In two patients no urine samples for determinations of albumin, IgG, transferrin, and β2M were collected.

The percentual decrease of proteinuria was defined as the difference of the protein excretion on the control day and NP3,

divided by proteinuria on the control day. Filtration fraction [FF] was defined as [GFR/ERPF]*100%. Renal blood flow was calculated as ERPF/[1-Ht]. The fractional excretion [FE] of a substance was defined as the renal clearance of that substance divided by the GFR and expressed as a percentage.

Values are given as means [±SEM]. When a non-normal distribution applied medians [interquartile range [IQR]] are given. For statistical analysis Student's t-test and Wilcoxon's sign rank test were used when appropriate. Linear regression was calculated using Spearman's correlation coefficient. A P value of less than 0.05 was considered significant.

The study protocol was approved by the Hospital Ethics Committee. All patients gave informed consent.

RESULTS.

Alternate-day prednisone treatment caused a suppression of endogenous cortisol production on the non-prednisone days, cortisol levels averaging 0.27 ± 0.04 mmol/l on the control day [C] and 0.10 ± 0.02 mmol/l on the third non-prednisone day [NP3; $p<0.01$]. Values of serum creatinine, serum sodium, and serum total protein were not different on the study days. However, hematocrit decreased slightly but significantly by on average -0.02±0.01 l/l [C:0.40 ± 0.02 l/l; NP3:0.38 ± 0.02 l/l; $p<0.05$].

After start of treatment we observed the typical fluctuating pattern of proteinuria. The changes in protein excretion could largely be explained by a decreased proteinuria on non-prednisone days. Urinary total protein excretion decreased from 6.1 mg/min [IQR 3.2-9.8 mg/min] at baseline to 2.5 mg/min [IQR 1.0-7.7 mg/min] on NP3 [$p<0.01$]. Values in the individual patients are shown in Fig. 1. Proteinuria decreased in all but one patient [no.8], percentual decrease averaging -45 ± 8% [range +20% to -94%].

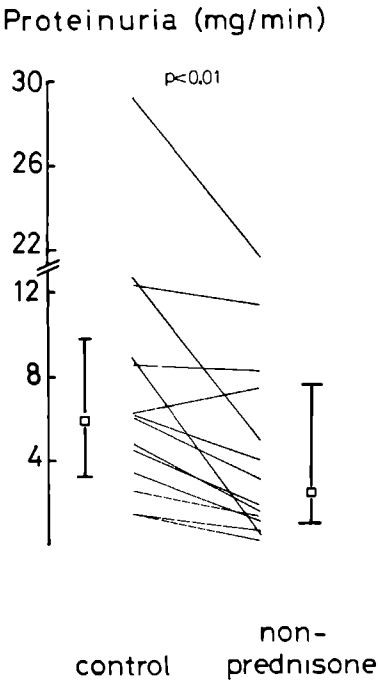

Figure 1. Proteinuria on the control day and on the third non-prednisone day. Data of the individual patients and medians [interquartile range] are shown.

Table II.Renal function parameters.

	Control	Non-prednisone	P-value
Urine flow [ml/min]	5.7 ± 0.9	6.5 ± 0.8	ns
UV_{Na} [μmol/min]	102 ± 16	100 ± 17	ns
ECC [ml/min]	112 ± 14	103 ± 11	p=0.08
GFR [ml/min]	75 ± 10	70 ± 8	p=0.10
ERPF [ml/min]	517 ± 71	516 ± 66	ns
RBF [ml/min]	893 ± 122	861 ± 121	ns
FF [%]	14.5 ± 0.8	13.5 ± 0.9	p<0.05

Abbreviations: UV_{Na}=urinary sodium excretion. ECC=endogenous creatinine clearance. GFR=glomerular filtration rate. ERPF= effective renal plasma flow. RBF=renal blood flow. FF=filtration fraction.

concomitant values of renal function parameters are given in Table II. Urinary flow rate and urinary sodium excretion were not different. Although ECC, GFR, ERPF, and RBF did not change significantly, filtration fraction fell slightly but significantly by -7 ± 2% [$p<0.05$].

The percentual decrease of proteinuria was significantly correlated with baseline GFR [Fig. 2; $r=0.75, p<0.01$], indicating that the decrease of proteinuria was most prominent in patients with well-preserved renal function.

The decrease of urinary protein excretion on NP3 was not restricted to a specific protein. Urinary excretion of all proteins that we measured decreased significantly [Table III].

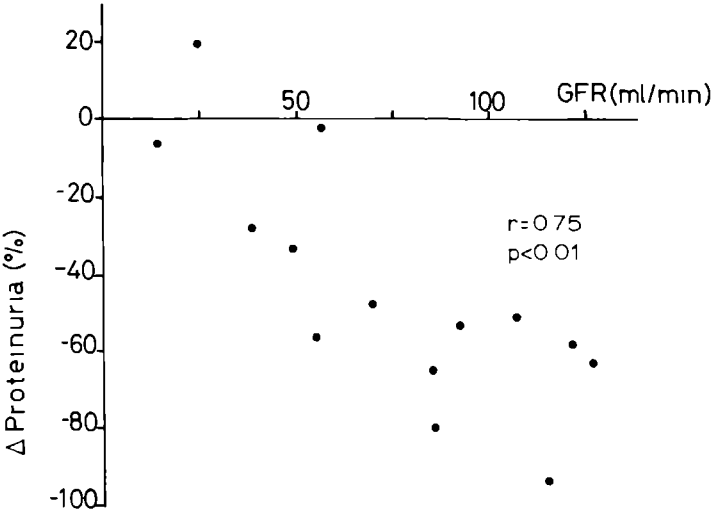

Figure 2. Relationship between the percentual decrease of proteinuria and baseline glomerular filtration rate [GFR].

Table III. Serum concentrations and urinary excretion rates of albumin, IgG, transferrin, and β2-microglobulin on the control day and the third non-prednisone day.

	Control	Non-prednisone	P-value
Serum:			
albumin [g/l]	20.4 ± 2.1	21.2 ± 1.9	ns
IgG [g/l]	3.9 [2.9-7.0]	3.4 [2.5-6.9]	p<0.01
transferrin [g/l]	1.9 ± 0.2	2.0 ± 0.2	ns
β2-microglobulin [mg/l]	2.7 ± 0.3	2.4 ± 0.3	p<0.02
Urinary excretion:			
albumin [mg/min]	3.5 [2.1-6.5]	2.1 [1.1-4.4]	p<0.01
IgG [mg/min]	0.08 [0.05-0.34]	0.04 [0.01-0.16]	p<0.01
transferrin [mg/min]	0.33 [0.16-0.62]	0.18 [0.03-0.32]	p<0.01
β2-microglobulin [μg/min]	0.32 [0.15-6.51]	0.26 [0.14-3.56]	p<0.01
Fractional excretion:			
albumin [%]	0.28 [0.10-0.70]	0.17 [0.06-0.47]	p<0.01
IgG [%]	0.36 [0.08-1.2]	0.12 [0.04-0.85]	p<0.01
transferrin [%]	0.27 [0.07-0.69]	0.16 [0.02-0.46]	p<0.01
β2-microglobulin [%]	0.17 [0.10-5.0]	0.15 [0.10-3.22]	p<0.05
Selectivity index	0.16 [0.10-0.27]	0.16 [0.10-0.27]	ns

Values are given as means [±SEM] or median [IQR].

The percentual decreases of the urinary excretion of these proteins are depicted in Fig. 3. The average decrease of IgG excretion [-60 ± 5%] exceeded the decrease of albumin excretion [-39 ± 9%; p=0.06], and of β2M [-24 ± 10%; p<0.01]. However, these differences are to some extent explained by the different changes in serum concentrations of the respective proteins [Table III], IgG and β2M being significantly lower on NP3. If the changes in GFR and serum concentrations are taken into account by calculating fractional excretions, percentual decreases of albumin, transferrin, and IgG excretion are comparable [Table III]. As a consequence selectivity of proteinuria remained unchanged. Fractional excretion of β2M [-12±10%] was reduced less than fractional excretions of IgG [-52±6%;p<0.01], transferrin [-52±7%; p<0.02], and albumin [-40 ±8%;p=0.06].

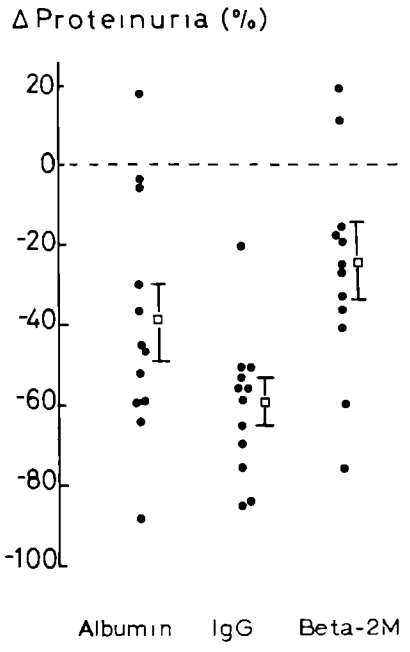

Figure 3.
Percentual decreases of urinary excretion of albumin, IgG, and β2-microglobulin. IgG vs. β2-microglobulin: $p<0.01$. Albumin vs. β2-microglobulin: $P=0.06$.

DISCUSSION

Our study demonstrates that during treatment of patients with high-dose prednisone on alternate days, proteinuria on non-prednisone days is decreased when compared to pre-treatment values. Our study extends recent observations on the fluctuating pattern of proteinuria during alternate-day prednisone therapy [3,6]. When we calculated the ratio of protein excretion rates on prednisone and non-prednisone days from these previous studies we found that proteinuria was a factor 1.5-2.0 higher on prednisone days [3,6]. In the present study the decrease of proteinuria on a non-prednisone day was compared with pre-treatment values and was found to average 45%, i.e. a ratio of 1.6. Thus, the observed changes in proteinuria during alternate-day prednisone therapy are predominantly determined by a decreased proteinuria on non-prednisone days, and to a lesser degree by a corticosteroid-induced increase of proteinuria on prednisone days.

Although our study was not controlled, it is unlikely that the decreases of proteinuria on non-prednisone days occurred spontaneously. In hospitalized patients only slight variations of proteinuria, occurring at random, are found [8]. Furthermore, although circadian rhythms of proteinuria exist, our observations would require the existence of a 48h-rhythm, and in detailed studies on the rhythmicity of proteinuria such a rhythm was not noted [9]. It is also unlikely that the decreases of proteinuria on non-prednisone days is explained by an instantaneous therapeutic effect of prednisone. First, treatment related reductions of proteinuria, if at all, are seldom observed in the first months after treatment start [10]. Secondly, we observed no further diminution of proteinuria on prednisone or non-prednisone days in patients studied during longer periods [3]. Thirdly, in most of the patients of the present study no sustained decrease of proteinuria was observed after the end of treatment.

Several mechanisms might explain the observed decrease in proteinuria: changes in systemic and renal hemodynamics, changes in tubular protein reabsorption, or changes in the permselectivity characteristics of the glomerular basement membrane [11]. Although we did not systematically measure blood pressure during the renal function measurements, we observed no difference in blood pressure measurements performed in our patients on prednisone and non-prednisone days. Kumagai et al. also did not observe differences in blood pressure between prednisone and non-prednisone days [6]. From this we conclude that changes in systemic hemodynamics probably do not contribute to the observed effects of alternate day prednisone therapy.

With regard to renal hemodynamics, we did not observe major alterations in glomerular filtration rate or renal blood flow. Filtration fraction decreased significantly, however. These results are in full agreement with the Japanese study, demonstrating unchanged GFR and ERPF, but a considerable decrease of

FF on non-prednisone days as compared to prednisone days [6]. This decrease of filtration fraction probably indicates a decrease in glomerular capillary pressure, which could at least partly explain the decrease in proteinuria. The fact that the change in filtration fraction is relatively minor [-7%] as compared with the change in proteinuria [-45%] would suggest that other factors must be involved. However, one should be careful to draw such a conclusion, since in human beings measurements of renal hemodynamics are not precise enough to detect changes in glomerular capillary pressure, especially when changes in ultrafiltration coefficient or renal blood flow distribution would occur concurrently.

We have used urinary β2M excretion as a marker of tubular protein reabsorption [12]. Under normal circumstances up to 99% of filtered β2M is reabsorbed by the proximal tubules. Tubular reabsoption of albumin is less efficient. After blockade of tubular protein reabsorption the relative increase of urinary β2M excretion exceeds the relative increase of urinary albumin excretion severalfold [13]. In the present study fractional excretion of β2M was altered less during alternate day prednisone therapy, which argues against important changes in tubular protein reabsorption. Also the magnitude of the effect makes an effect through altered tubular protein reabsorption less likely. In case of a defect in tubular protein transport, proteinuria hardly ever exceeds 2 g/24h, which is taken as evidence that tubular transport is limited. Certainly, we cannot completely exclude that tubular transport capacity is increased in patients with a nephrotic syndrome. In fact, recent studies in rabbits have demonstrated that tubular transport of albumin is not readily saturable , and has a high capacity [14].

In recent years it has become evident that the transport of macromolecules through the glomerular filter is determined by molecular size and configuration [size-selectivity] and by molecular charge [charge-selectivity]. We have studied urinary

excretions of proteins of different size and charge such as albumin, transferrin, and IgG. The decreases of urinary excretion of these proteins were comparable, favouring a predominant change in glomerular size-selectivity. This again is in close agreement with the results of Kumagai et al. who studied the fractional clearances of neutral dextrans to detect changes in glomerular permeability [6], and observed that the fractional clearances of dextran molecules with a molecular radius greater than 3.6 nm were significantly lower on the non-prednisone day.

Thus, proteinuria is decreased on non-prednisone days during alternate day steroid therapy. Our and Kumagai's data suggest that the decreased proteinuria is related to a decreased capillary pressure and a decreased glomerular permeability. How could alternate-day prednisone therapy influence proteinuria? Based on data reported in the literature it is tempting to speculate on the role of cortisol in proteinuria. Adrenalectomy reduces the basal proteinuria in normal and spontaneously hypertensive rats [15]. Similar reductions of proteinuria are seen in male rats after hypophysectomy [16]. Also, proteinuric responses to renin, angiotensin II and norepinephrine are completely inhibited by adrenalectomy or hypophysectomy [7,16,17]. In most studies proteinuria or proteinuric responses are normalized after administration of glucocorticoids but not mineralocorticoids [7,15-17]. Based on these studies glucocorticoids are thought to exert a permissive action on proteinuria. The exact mechanism of this effect is debated. In most studies the importance of hemodynamic factors is stressed, adrenalectomized rats demonstrating a low GFR and ERPF. If administration of corticosteroids is followed by normalisation of GFR, proteinuria is restored to normal [17]. However, Bauman did not observe major differences in renal hemodynamics between adrenalectomized rats treated with dexamethasone or aldosterone [7]. Since dexamethasone but not aldosterone restored proteinuria to normal he argued that corticosteroids might directly alter glomerular permeability.

Results of recent studies have stimulated the interest in the effects of corticosteroids on proteinuria. In the remnant-kidney model in the rat, a non-immunological model of progressive renal failure, chronic administration of pharmacological doses of methylprednisolone enhanced proteinuria and accelerated the development of glomerular sclerosis [18]. These detrimental effects of chronic steroid administration were attributed to an increased glomerular capillary pressure. In this respect chronic steroid administration closely resembles daily high protein feeding [19]. Our findings demonstrate that during alternate-day prednisone treatment proteinuria and possibly also glomerular capillary pressure are intermittently decreased. In view of the fact that intermittent high protein feeding has less damaging potential in the above-mentioned rat model, it is tempting to speculate that intermittent administration of steroids might prove less vulnerable to the kidneys than daily administration. Evidence for a role of cortisol production in progressive renal insufficiency in humans was put forward in a recent study in patients with renal failure [20]. The excretion of 17-hydroxycorticosteroids proved a major determinant of the rate of progression. Patients with a relatively low production of glucocorticoids showed a slower progression rate. This study indicates that steroids at physiological levels might influence glomerular damage. Our observation that proteinuria is reduced at moderately decreased plasma cortisol levels seems compatible with a role for steroids in the process of continuing glomerular damage.

In conclusion: we have demonstrated a decreased proteinuria on non-prednisone days during alternate-day prednisone therapy. Suppression of endogenous cortisol production resulting in a decreased glomerular capillary pressure and a decreased glomerular permeability may be the primary determinants of this effect. Further studies on the effects of corticosteroids on proteinuria might open new ways to treatment of proteinuria and progressive renal failure.

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CHAPTER IX

ANALYSIS OF SHORT-TERM ALTERNATE-DAY PREDNISONE THERAPY IN PATIENTS WITH MEMBRANOUS GLOMERULONEPHRITIS

J.F.M. Wetzels, A.J. Hoitsma, R.A.P. Koene

ABSTRACT

We have analysed the effects of treatment with prednisone, 125-150 mg on alternate days for eight weeks, in 34 patients [28 male, 6 female] with biopsy-proven idiopathic membranous glomerulonephritis. Median duration of disease at the start of treatment was two months [range 0-146 months] and follow up after start of treatment averaged 52 months [range 12-108 months]. Thirteen patients [38%] reached a partial remission of proteinuria, which was followed by a complete remission in six [18%]. After five years 25% of the patients had died or had reached end-stage renal disease. Renal function deteriorated in none of the women. In contrast, 15 men [54%] developed renal insufficiency, nearly all within two years after start of treatment. Patients developing renal insufficiency were characterized by impaired renal function, higher proteinuria, lower protein selectivity, and higher blood pressure at the start of the treatment. Retrospectively, an increased urinary excretion of the low molecular weight protein β2-microglobulin was a good discriminative marker of subsequent deterioration of renal function. In conclusion: our results indicate that prednisone treatment according to the abovementioned schedule is of doubtful significance in patients with membranous glomerulonephritis. New immunosuppressive treatment regimens might best be restricted to male patients in whom prognostic factors point to a high likelihood of progressive renal failure.

INTRODUCTION

Membranous glomerulonephritis is the most common cause of the nephrotic syndrome in adults [1]. The natural course of idiopathic membranous glomerulonephritis is quite variable. In the long run about half of the patients reach a complete or partial remission of proteinuria, whereas the other half develop progressive renal insufficiency [2]. It is debatable, whether treatment with corticosteroids or other immunosuppressive drugs can alter the course of the disease [3-5]. In 1979 the Collaborative Study of the Adult Idiopathic Nephrotic Syndrome reported beneficial effects of short-term alternate-day prednisone treatment [6]. Lately, the efficacy of prednisone treatment has been questioned [7], and more promising results were obtained using the immunosuppressive drug chlorambucil [8]. Since 1980 we have been treating patients with idiopathic membranous glomerulonephritis with alternate-day prednisone according to the protocol of the abovementioned study [6]. We have analysed the course of disease in these patients and have tried to identify factors which could predict subsequent development of renal insufficiency.

PATIENTS AND METHODS

Since 1980 we have investigated the acute effects of prednisone on proteinuria in patients with a nephrotic syndrome [9-11]. In these studies 34 patients with a biopsy-proven idiopathic membranous glomerulonephritis were included. They were treated with prednisone, 125-150 mg on alternate days for eight weeks, and were regularly followed thereafter. We have reviewed the clinical records of these patients and recorded the relevant data. One patient [no 6] was lost to follow-up five years after start of treatment.

In half of the patients detailed measurements of renal function were done at the start of treatment. Renal clearances of inulin and PAH were used as markers of glomerular filtration rate

[GFR] and effective renal plasma flow [ERPF] respectively. At the time of these renal function measurements, blood and urine samples were drawn for the measurements of albumin, transferrin, IgG, and β2-microglobulin. To keep urinary pH above 6.0, which is necessary for correct measurement of urinary β_2-microglobulin, most patients were pretreated with sodium bicarbonate.

Albumin, transferrin, and IgG were measured by immunonephelometry using specific antibodies raised in rabbits. Serum and urinary β_2-microglobulin were measured using a commercially available radioimmunoassay [Pharmacia]. All other labatory parameters were measured using standard semi-automated techniques. Selectivity of proteinuria was calculated as the ratio of the clearances of IgG and transferrin. Proteinuria was measured in a 24 h urine sample or, if not available, calculated from the protein/creatinine ratio determined in a random urine sample. A complete remission of proteinuria was defined as a decrease to less than 0.2 g/24h, a partial remission as a decrease to less than 2.0 g/24h.

Differences between groups were analysed using a non-parametric Wilcoxon test. Values are given as means [± SEM] or medians [interquartile range] when appropriate.

RESULTS

We have studied 34 patients [28M, 6F] with idiopathic membranous glomerulonephritis. The median interval between renal biopsy and start of prednisone treatment was 2 months [range 0-146 months]. In three quarter of patients treatment was started within eight months after biopsy. Clinical data at the start of treatment are given in table I. In all patients the treatment course was completed. The evolution of proteinuria is depicted in figure 1. Overall 13 patients [10M, 3F] reached a partial remission of proteinuria, which was followed by a complete remission in six [18%]. In three patients a complete remission

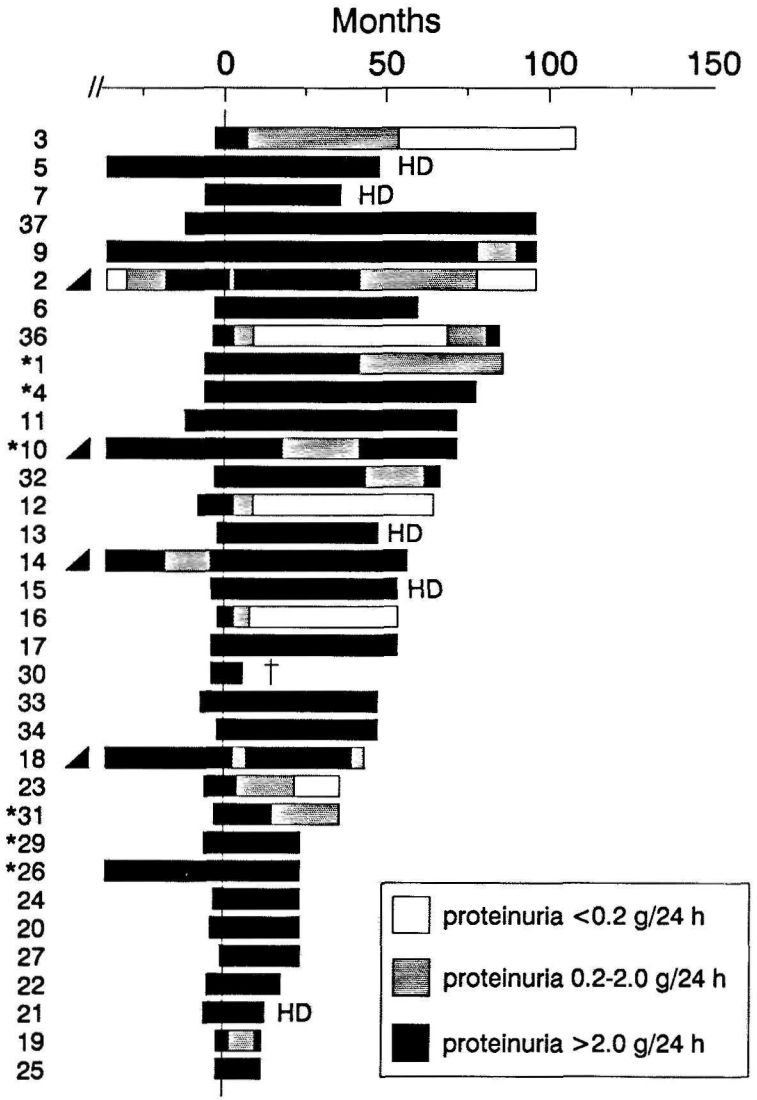

Figure 1: Evolution of proteinuria before and after the start of prednisone treatment [at 0 months]. Patient numbers are indicated at the left. Female patients are marked with an asterisk. HD=hemodialysis. One patient died during follow up [†].

Table I. Clinical data at start of prednisone treatment.

	Men [n=28]	Women [n=6]
Age [years]	45 [±3]	39 [±5]
Blood pressure [mmHg]		
systolic	145 [±2]	156 [±8]
diastolic	85 [±2]	87 [±3]
Serum creatinine [μmol/l]	92 [81-124]	89 [71-113]
Serum albumin [g/l]	24.7 [±1.3]	25.5 [±1.5]
Proteinuria [g/24h]	7.0 [5.5-13]	9.0 [6.5-15.5]
ECC [ml/min/1.73m^2]	89 [82-113]	90 [65-121]

Abbreviation: ECC = endogenous creatinine clearance. Values are given as means [±SEM] or medians [interquartile range].

was reached within one year after start of treatment. It is evident from the figure that most patients have a persistent proteinuria of more than 2.0 g/24h. Persistence of an evident nephrotic syndrome [i.e proteinuria >3.5 g/24h and serum albumin <30 g/l] is rare however, occurring in 9 of 29 patients at two years and in only one patient at four years after the start of treatment. The course of renal function is depicted in figure 2. During follow-up, five patients reached end stage renal disease, all within five years after start of treatment, and one patient died because of a myocardial infarction. Women showed a good prognosis, since in none renal function decreased. In contrast, deterioration of renal function [i.e serum creatinine >130 μmol/l] developed in 15 of 28 male patients [54%]. Twelve of these patients could be identified within two years after start of therapy. When comparing patients with stable renal function [group I] and those with deteriorating renal function [group II], differences are evident [table II]. Patients progressing to renal insufficiency are characterized by a higher blood pressure, an elevated serum creatinine, more proteinuria and a lower selectivity of proteinuria. In the subgroup of patients in whom more detailed studies of renal function and proteinuria were done, a similar pattern occurred, which was reflected in differences in GFR [78±7 vs 45±7 ml/min/1.73m^2; p<0.01], albuminuria [3.3±0.8 vs 7.1±1.4 mg/min; p <0.05], serum transferrin [2.4±0.3 vs 1.5±0.2 g/l; p <0.05],

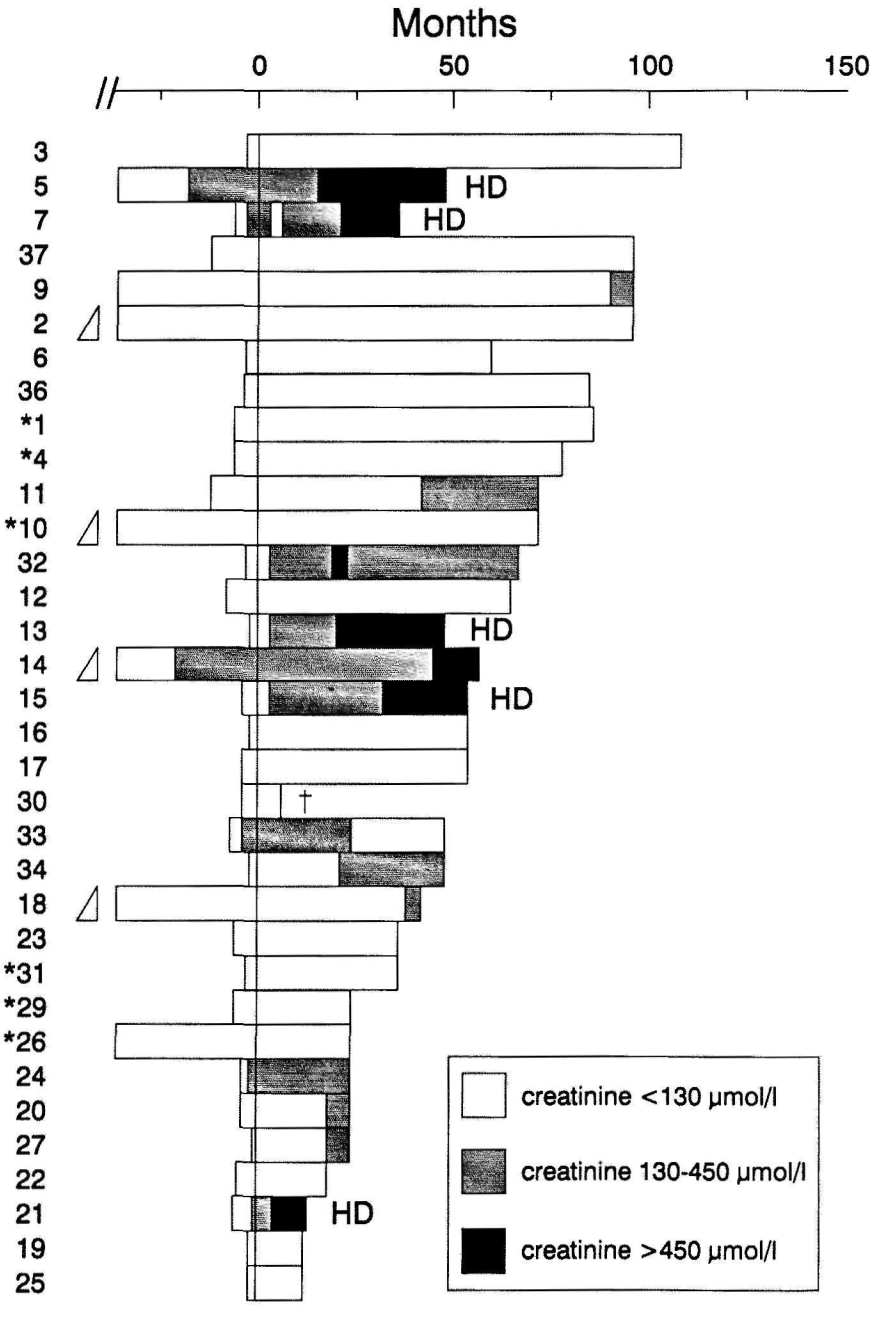

Figure 2: Course of renal function as reflected by serum creatinine concentration. For explanation see figure 1.

Table II. Clinical data in men with and without renal insufficiency during follow-up

	Group I [n=13]		Group II [n=15]		P-value
Age [years]	44	[4]	45	[4]	N.S.
Blood pressure [mmHg]					
systolic	139	[3]	150	[3]	P<0.05
diastolic	82	[2]	89	[2]	P<0.01
Serum creatinine [μmol/l]	82	[79-98]	118	[95-150]	P<0.01
ECC [ml/min]	105	[84-131]	81	[61-95]	P<0.01
Serum albumin [g/l]	26.8	[1.6]	22.9	[1.8]	N.S.
Proteinuria [g/24h]	6.0	[5.3-7.3]	10.2	[6.7-15.6]	P<0.02
Selectivity index*	0.12	[0.02]	0.24	[0.04]	P<0.05

Abbreviations: ECC=endogenous creatinine clearance. Values are given as means [SEM] or medians [interquartile range]. Group I represents patients with stable renal function, group II patients with renal insufficiency.
* Values recorded in 10 and 9 patients respectively.

and serum β2-microglobulin [1.9±0.1 vs 3.3±0.4 mg/l]. In addition group II patients nearly all had increased urinary excretion of β2-microglobulin [group I: 147ng/min [IQR 104-255] group II: 2806 ng/min [IQR 760-12209]; p<0.01]. Most of these differences can be attributed to the initially already impaired renal function in group II patients. When we analysed patients with an initial endogenous creatinine clearance [calculated from serum creatinine and 24h creatinine excretion] above 80 ml/min/1.73m^2 , urinary β_2-microglobulin excretion was most discriminative in predicting subsequent renal function deterioration [Fig.3]. In all but two patients with a serum creatinine above 130 μmol/l, renal function declined progressively. In the two patients without progression an improvement of renal function was observed after treatment with chlorambucil and prednisone as described previously [12]. Side effects of treatment were infrequent:steroid diabetes [n=1], sinusitis [n=1], erysipelas [n=1], and perforated sigmoid in a patient with known diverticular disease.

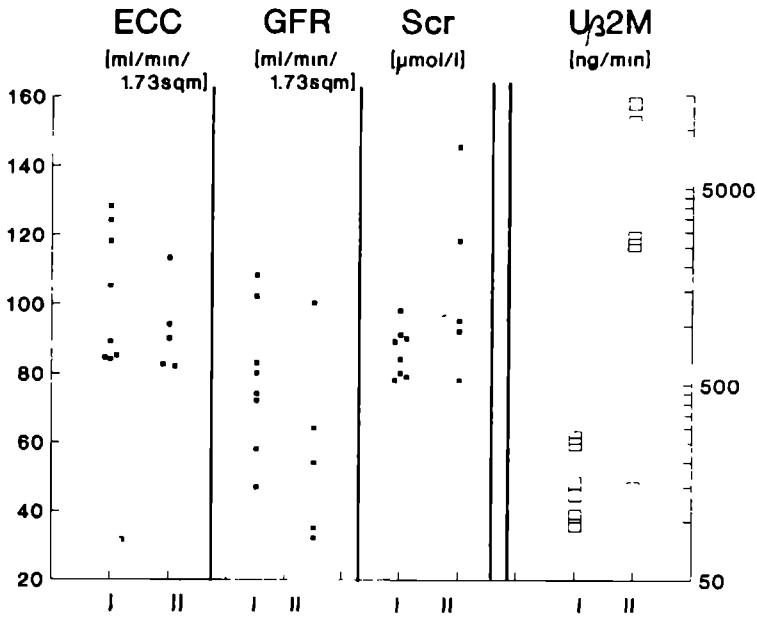

Figure 3: Comparison of laboratory parameters in patients with initial ECC higher than 80 ml/min/1.73m^2. Patients are divided according to course of renal function. Group I represents patients with stable renal function, group II patients with renal insufficiency during follow-up. ECC=endogenous creatinine clearance; GFR=glomerular filtration rate (inulin clearance); Scr=serum creatinine; Uβ2M=urinary β2-microglobulin excretion.

Idiopathic membranous glomerulonephritis follows a variable, rather indolent course [reviewed in chapter II]. Up to this moment no consensus exists regarding the treatment of these patients [3-5]. In 1979, the results of the first controlled trial, involving a sufficient number of patients, were reported [6]. Patients were treated with alternate-day prednisone for eight weeks. Results of the trial were promising, especially with respect to the prevention of deterioration of renal function. Recently, controlled trials published in full [7] or in abstract form [13] could not confirm the beneficial effects of prednisone monotherapy in idiopathic membranous glomerulonephritis. However, combined treatment with prednisone and chlorambucil proved very effective [8], even in patients with deteriorating renal function [14, and reviewed in chapter II and X]. It is, however, not clear whether this difference was caused by the addition of chlorambucil to the treatment or by the fact that the course was started with three intravenous pulses of high dose methylprednisolone.

In view of the recent discussion regarding prednisone treatment in patients with idiopathic membranous glomerulonephritis, we have analysed the course of the disease in our patients who had been treated with short-term alternate-day prednisone. The clinical characteristics of our patient group recorded at the start of prednisone treatment fit well with those reported in the literature, patients being predominantly male, 85% having a nephrotic syndrome, and 20% an impaired renal function at the start of treatment.

Our results confirm that membranous glomerulonephritis has a favourable course in women [15, and chapter II]. Male patients do clearly less well: in more than half of these patients renal function deteriorated during follow-up. In agreement with data in the literature most of the patients with deteriorating renal function could be identified within two years after treatment

start.

Overall 38% of our patients reached a partial or complete remission of proteinuria, whereas 45% developed renal insufficiency. These results are comparable with the results reported in untreated patients, where the reported frequency of partial and complete remission varies from 18-65%, and the percentage of patients with evidence of renal function deterioration ranges from 19-52% [chapter II]. Certainly, since our study is uncontrolled our data do not permit to exclude any beneficial effect of short-term prednisone treatment. However, in any event the results indicate that a large number of patients do not benefit from this treatment regimen.

Some clinical characteristics can apparently help to identify patients who are at risk for developing renal insufficiency at an early stage. These factors are: male sex, severe proteinuria, non-selective proteinuria, and impairment of renal function. In addition, urinary excretion of the low molecular weight protein β_2-microglobulin may be a useful discriminative marker in this respect.

In a recent controlled study a combination of chlorambucil and prednisone was very effective in patients with idiopathic membranous glomerulonephritis [8]. A similar treatment regimen was also effective in patients with deteriorating renal function [14]. Using a similar regimen we were able to reverse the decrease of renal function in two patients [12]. If these results are confirmed, such an agressive treatment could be reserved for patients at high risk of developing progressive renal failure, and treatment could be safely withheld from patients who probably will never develop renal failure.

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CHAPTER X

CHLORAMBUCIL IN STEROID-RESISTANT MEMBRANOUS GLOMERULONEPHRITIS

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ABSTRACT

We have studied the effects of chlorambucil in eight patients with idiopathic membranous glomerulonephritis, who had been treated with high doses of prednisone for at least three months, but showed persisting nephrotic syndrome and/or deteriorating renal function. All were men and their mean age was 48 years [range 17-64 y]. Chlorambucil was given in a dose of 0.2 mg/kg/day in three periods of four weeks, separated by two week intervals during which the patients were treated with prednisone in a dose of 125 mg on alternate days. All patients were followed for at least 24 months. For the whole group of patients the rise of mean serum creatinine was interrupted, proteinuria decreased, and serum albumin increased after start of the chlorambucil treatment. However, these beneficial effects were most prominent at six months and not sustained in all patients. In two patients who entered the study with stable renal function, this remained unchanged. Six patients entered the study with evidence of deteriorating renal function. In five renal function improved or stabilized, but this response was sustained in only three. Side effects of chlorambucil treatment were anorexia and nausea [n=3], itching [n=2], and exanthema [n=1]. Furthermore, in all but one patient the dosage of chlorambucil had to be reduced because of leukocytopenia and thrombocytopenia. We conclude that chlorambucil may have beneficial effects in some patients with membranous glomerulonephritis who did not respond to prior treatment with corticosteroids.

INTRODUCTION

Membranous glomerulonephritis is the most common glomerular disease underlying the nephrotic syndrome in adults [1]. The clinical course of the disease is rather unpredictable, complete remissions occurring spontaneously in about a quarter of untreated patients, whereas up to 50% show a steady decline of renal function [2,3]. Whether drug therapy can alter the course of the disease is still a matter of controversy [4-6]. Short-term high-dose alternate-day prednisone therapy was used in a controlled, prospective study conducted more than 10 years ago and seemed effective in preventing progressive renal failure [7]. These results have been questioned however, and could not be confirmed in more recent trials [8,9]. In a recent study in patients with well-preserved renal function promising results were obtained using a six month course of alternating monthly cycles of chlorambucil and prednisone [10]. Up to 75% of treated patients reached a complete or partial remission of proteinuria, and only a minority showed evidence of renal function deterioration. We have studied the effect of chlorambucil in patients with membranous glomerulonephritis who had been treated with prednisone, but who showed persisting nephrotic syndrome and/or a decline of renal function.

PATIENTS AND METHODS

We have studied eight men with biopsy-proven idiopathic membranous glomerulonephritis. All had been treated with high-dose prednisone for at least three months, but showed persistent nephrotic syndrome and/or deteriorating renal function. Clinical data of the patients are given in Tables I and II. Patients were treated with chlorambucil and prednisone. In view of the earlier exposure to steroids we slightly modified the treatment regimen of Ponticelli [10]. In particular we omitted the three initial pulses of one gram of methylprednisolone and we reduced the prednisone treatment periods from four to two weeks. Chlorambucil was prescribed in a dose of 0.2 mg/kg/day

Table I. Patient characteristics at the start of chlorambucil treatment.

Patient no.	Age [yr]	Serum creatinine [μmol/l]	Proteinuria [g/24h]	Blood pressure [mmHg]	Medication
1	53	80	8.6	120/ 70	Ac
2	45	176	8.3	130/ 85	Al,P,E,T
3	34	136	6.2	115/ 85	-
4	64	161	10.6	160/100	Ald,F,R
5	17	511	3.2	180/115	A,H,T
6	62	175	22.3	180/ 90	Ac,F
7	57	126	12.4	158/ 70	-
8	50	262	9.1	170/105	F,M

Abbreviations: A=acebutolol, Ac=acenocoumarol, Al=allopurinol, Ald=aldactone, E=endralazine, F=furosemide, H=hydralazine, M=metoprolol, P=pindolol, R=ranitidine, T=thiazide.

Table II. Patient characteristics [continued].

Patient no.	Renal biopsy findings Grade	Tubulointerstitial lesions	Duration of previous treatment [months]	Interval to start of chlorambucil [months]	Follow up [months]
1	II-III	+	3	4	24
2	II	-	3	7	36
3	I	-	31	1	36
4	II	++	3	5	24
5	II-III	+	3	21	36
6	II	+	3	1	36
7	I	-	3	32	30
8	I-II	++	12	15	36

Staging of the biopsies was according to Ehrenreich [18]. Biopsies were scored quantitatively for the presence of tubulo-interstitial changes.

in three periods of four weeks, separated by two week intervals during which the patients were treated with 125 mg of prednisone on alternate days. In case of leucopenia or thrombocytopenia the dosage of chlorambucil was reduced. Patients were seen at the outpatient clinic at regular intervals. Follow-up was at least 24 months in each patient.

Laboratory parameters were measured using standard techniques. A complete remission was defined as a reduction of proteinuria to less then 0.2 g/24h, a partial remission as a reduction of proteinuria to less then 2.0 g/24h.

For statistical analysis we used Student's t-test and Wilcoxon's test when appropriate.

RESULTS

For the whole group of patients the rise in mean serum creatinine was interrupted, proteinuria decreased and serum albumin increased after start of chlorambucil treatment [Table III]. The time course of proteinuria in individual patients is given in Fig 1. It is evident that proteinuria decreased in all patients after start of chlorambucil and remained below baseline values in all patients but one. However, no patient reached a complete remission of proteinuria, whereas a partial remission at the end of follow-up was noted in only one patient. The time course of renal function as reflected by the serum creatinine is given in Fig 2. Two patients entered the study with normal and stable renal function. In these patients no change in renal function was observed. Six patients entered the study with evidence of deteriorating renal function. In five of them renal function improved or stabilised for several months after start of therapy. However, a clear sustained improvement of renal function at the end of follow up was observed in only two and a stabilization in one. One patient progressed to end-stage renal disease within three years after start of treatment.

Table III. Biochemical parameters before and after chlorambucil treatment.

Laboratory parameters	Period of observation (months)			
	-12	0+	6	24
Serum creatinine [μmol/l]	136 ± 46*	203 ± 135	176 ± 85	222 ± 126
Serum albumin [g/l]	30 ± 8	28 ± 9	35 ± 7*	35 ± 7*
Proteinuria [g/24h]	12.4 ± 4.8	10.3 ± 5.5	4.4 ± 3.3*	6.0 ± 3.6

Values are given as means ± SD. +; start of chlorambucil treatment.
* $p<0.05$ compared to values at start of chlorambucil treatment.

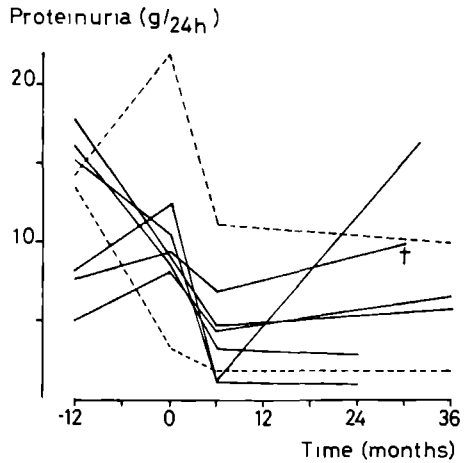

Figure 1. Time course of proteinuria before and after start of chlorambucil treatment at t= 0 months. Patients showing an improvement of renal function are depicted by an interrupted line. † = start of hemodialysis.

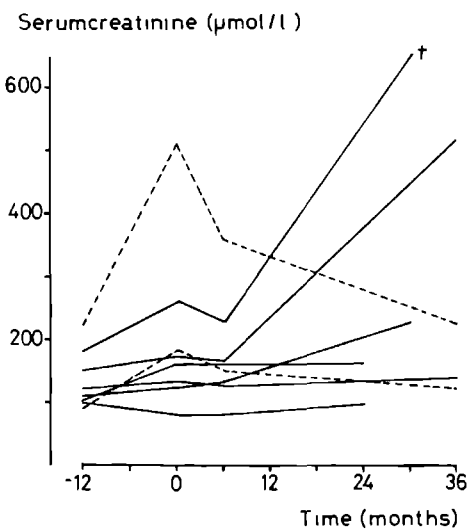

Figure 2. Time course of serum creatinine before and after start of chlorambucil treatment. For explanation see figure 1.

Side effects of chlorambucil treatment were anorexia and nausea [n=3], itching [n=2], and exanthema [n=1]. Furthermore, in five patients the dosage of chlorambucil had to be reduced to 0.1 mg/kg/day and in two the treatment had to be stopped after six and eight weeks because of leukocytopenia and/or thrombocytopenia.

DISCUSSION

Idiopathic membranous glomerulonephritis is characterized by an unpredictable course. Although nearly half of untreated patients will ultimately develop renal insufficiency, still 30-50% of patients show spontaneous, complete or partial remissions of proteinuria, while renal function remains stable [2,3]. Factors associated with a less favourable outcome are male sex, older age, nephrotic syndrome, impairment of renal function, the presence of tubulo-interstitial changes, histological grade III/IV, and haplotype B18-DR3-BfF1 [2,10,11-13]. Treatment of idiopathic membranous glomerulonephritis is controversial [4-6], which can be attributed to the scarcity of controlled, prospective trials. Furthermore in most studies, prospective as well as retrospective, follow-up of patients is rather short, and populations studied are quite heterogeneous.

In 1979 the results of a well-conducted, prospective controlled study were reported, showing beneficial effects of short-term treatment with high-dose prednisone on alternate days [7]. Although treated patients at the end of follow-up did not have more remissions of proteinuria, a decline of renal function was prevented. However, these results have not been confirmed in more recent trials [8,9], and therefore the beneficial effect of prednisone treatment remains questionable. In 1984 favourable effects were reported of treatment with chlorambucil and prednisone [10]. Up to 75% of treated patients reached a complete or partial remission of proteinuria, and nearly all demonstrated stable renal function. We have studied the effects of a similar treatment regimen in patients with persisting

nephrotic syndrome and/or deteriorating renal function despite treatment with high-dose prednisone. All our patients were men, which can be explained by the higher incidence and the less favourable outcome of membranous glomerulonephritis in men. The treatment regimen resulted in a clearcut decrease of proteinuria parallelled by an increase in serum albumin concentration. However, no patient reached a complete remission and in only one a sustained partial remission was noted. With respect to renal function, the effects of treatment were less evident. Overall, no change in serum creatinine was noted. In two patients who entered the study with stable and normal renal function, this remained unchanged. In six patients who entered the study with evidence of deteriorating renal function, a sustained improvement was noted in two, and a stabilization in one, whereas the other three showed further decline of renal function, leading to end stage renal disease in one of them. Side effects were frequent, and in all but one patient chlorambucil dosage had to be reduced because of leukocytopenia and/or thrombocytopenia.

To our knowledge thusfar only uncontrolled trials have been conducted in patients with deteriorating renal function [reviewed in chapter II]. The results of these studies indicate that renal function can improve in these patients, who, if left untreated, relentlessly progress to end stage renal disease. Three studies have used combined treatment of prednisone and chlorambucil [Table IV]. Excellent results have been obtained by Mathieson et al [14], who treated eight patients with deteriorating renal function using exactly the Ponticelli regime including the initial three pulses of one gram of methylprednisolone and chlorambucil in a dose of 0.2 mg/kg/day. In all eight patients proteinuria decreased, one reaching a complete and three a partial remission. Renal function improved in six, and stabilised in one. Although in their original article follow-up was rather short, averaging 11 months, the improvement was sustained after a mean follow up of 18 months [15]. In the study of Warwick and Boulton-Jones [16], seven pa-

Table IV. Summary of therapeutic trials using chlorambucil in patients with membranous glomerulonephritis and deteriorating renal function.

Ref.	Patients (no)	Sex M/F	Creatinine (μmol/l)	Follow-up (months)	Proteinuria CR	Proteinuria PR	Renal function IM	Renal function S	Renal function ESRD
14	8	7/1	194 [122-312]	18	1	3	6	1	1
16	7	7/0	300 [180-480]	11	1	1	1	3	0
This Study	6	6/0	235 [126-511]	33	0	1	2	1	1

Abbreviations: CR=complete remission, PR=partial remission, IM=improved, S=stabilised, ESRD=end stage renal disease.

tients were treated with monthly cycles of prednisone and chlorambucil. These authors, like we, did not use pulses of methylprednisolone, and used a lower dose of chlorambucil [0.12 mg/kg/day]. They noted an improvement of renal function in one patient and stabilization of renal function in three others. The results of the latter study are very similar to ours, and clearly less impressive than the results of Mathieson et al. What has caused these differences? In view of the small numbers of patients it might have occurred by chance. Alternatively, patient groups are somewhat different, renal function being more severely impaired in the patients of Warwick and Boulton-Jones, whereas our patients all had been unsuccesfully treated with prednisone. Lastly, differences in treatment regimens might be important. Although the recommended dosage schedule was different in the three studies, the average chlorambucil intake was quite similar, mainly because the development of leukocytopenia and thrombocytopenia necessitated dose reduction in our and Mathieson's study. The main difference in the treatment schedules was the use of intravenous pulses of methylprednisolone by Mathieson and colleagues. Although no firm evidence exists about the superiority of pulses methylprednisolone in the treatment of primary glomerulonephritis, the omission of these pulses in our and Warwick's study might explain the different results. In this regard it is interesting that Short and colleagues, who used pulses methylprednisolone

in addition to high dose oral prednisone were able to reverse the progression of renal failure in nine of 15 patients with deteriorating renal function [17].

Up to this moment no definite conclusions can be drawn from the studies mentioned. However, it is evident that patients with membranous glomerulonephritis and deteriorating renal function may respond to several different immunosuppressive regimens. If the results of the study of Mathieson et al. are confirmed in further trials it would allow us to limit immunosuppressive therapy to patients with progressive disease. In patients in whom the disease will follow an indolent course without progression to renal failure unnecessary treatment with its associated short-term adverse effects and its potential long-term hazards could be avoided.

APPENDIX

The following colleagues participated in the Study Group: Dr. R. Go, Nijmegen, Dr. G. Jordans, Enschede, Dr. R. v.Leusen, Arnhem, Dr. R. Schlattmann, Roosendaal, Dr. V. Verstappen, Venlo, and Dr. A. Woittiez, Almelo.

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SUMMARY AND DISCUSSION

In 1979, beneficial effects of prednisone treatment were reported in patients with membranous glomerulonephritis [1]. The authors advised to use an alternate day regimen, in order to reduce side effects. While treating patients with membranous glomerulonephritis according to this regime, we noted an increase of proteinuria on prednisone days [2]. These observations prompted further studies on this effect. The results of these studies form the main theme of this thesis. When studying the effects of prednisone on proteinuria some methodological questions arose, such as the influence of urine flow on proteinuria, the reliability of creatinine as a marker of glomerular filtration, and the possibility to measure the influence of the charge of the glomerular basement membrane on proteinuria. These questions were addressed in separate studies. Finally, because recent studies have questioned the efficacy of prednisone treatment in patients with membranous glomerulonephritis, we have analysed the course of the disease in our patients, who were treated with prednisone alone, or in combination with chlorambucil.

Methodological studies

Under normal living conditions both normal and pathologically increased urinary protein excretion rates are quite variable, and dependent on posture, exercise, diet, time of the day, and preciseness of urine sampling. In a clinical setting most of these factors can be controlled. Uncertainty existed on the influence of urinary flow rate on proteinuria. We have therefore studied the relation of protein excretion to urinary flow rate in patients with renal disease and proteinuria. At a urinary flow rate above 1.5 ml/min proteinuria is fairly constant and independent of diuresis. However, at flow rates below 1.0 ml/min proteinuria is diminished, which might result from an increased tubular protein reabsorption. During hemodynamic studies performed over a short period of time, it is not difficult to keep diuresis constant over 2.0 ml/min. However, when urine is sampled over longer periods e.g. 24 hours,

diuresis will hardly ever exceed 1.5 ml/min, i.e. 2.2 L/day. Therefore, it is important that urinary flow rate is accounted for when measuring proteinuria.

In recent years evidence has been gathered mostly from animal experiments showing that protein transport is determined not only by the size of the molecules involved but also by their charge. Information on the charge selective properties of the glomerular filter cannot easily be obtained in humans. It is well known that in normal serum two major amylase isomers exist, pancreatic-amylase [P-amylase] and salivary-amylase [S-amylase]. These iso-enzymes have an identical size [2.9 nm], but different charge, S-amylase [iso-electric point 5.9-6.4] being more anionic than P-amylase [iso-electric point 7.0]. We have investigated whether this difference could be used to obtain insight into the charge selective properties of the glomerular basement membrane in the clinical situation. In a pilot study we studied the clearances of these amylase iso-enzymes in healthy subjects and in patients with renal disease and variable proteinuria. In agreement with our understanding of the charge selective properties of the glomerular basement membrane we observed that in the healthy subjects renal clearance of neutral P-amylase exceeded the clearance of the anionic S-amylase. In patients with renal diseases clearance of S-amylase was relatively increased, consistent with a defect in the charge-selectivity properties of the glomerular capillary wall. Comparing patients with normal and abnormal tubular function no difference in the ratio of the fractional clearances of P-amylase and S-amylase was observed, which indicates that both iso-enzymes are processed by the tubules in the same way. Although these results suggest that it is worthwile to study further the possible usefulness of measuring the fractional clearances of P- and S-amylase as markers of glomerular basement membrane charge, some restrictions should be made. First, the inhibitor technique that we have used for measuring amylase iso-enzymes is rather insensitive, especially if the proportion of P-amylase in the urine exceeds 90%. In fact, as

mentioned, we could not determine urinary S-amylase in 11 of 63 subjects. Secondly, we cannot exclude that the inhibition characteristics of the inhibitor are influenced by uremic products in the patients' serum or urine. To circumvent these problems, we are currently investigating a technique using monoclonal antibodies that specifically inhibit S-amylase. Results thusfar are promising [3]. Further studies are planned to determine if we can find changes in the excretion patterns of S-amylase in diabetic patients, in whom glomerular basement membrane charge decreases at an early stage of the disease. Thusfar we have studied patients with microalbuminuria, and found no major differences in the excretion patterns of S- and P-amylase. This is somewhat disappointing, and might indicate that the method is not sensitive enough to detect minor changes in glomerular basement membrane charge.

The glomerular filtration rate [GFR] is an important parameter of renal function. In routine clinical practice creatinine is widely used as a marker of GFR. In recent years, the reciprocal of serum creatinine [1/Screat] has become popular for examining changes in renal function in time and for assessing the possible effects of therapeutic interventions. However, there are several pitfalls in the use of creatinine as marker of GFR. It is important to realise that serum creatinine and urinary creatinine excretion are partially dependent on creatinine derived from exogenous sources, especially meat. Furthermore, tubular secretion and metabolism may contribute substantially to creatinine disposal. The variability of these factors makes creatinine an unreliable marker of GFR, especially in clinical studies where relatively small changes in renal function have to be detected. A striking example of the problems that can arise when using 1/Screat to follow renal function in time comes from studies on the effects of protein restriction on the progression of renal failure. These studies have claimed that early protein restriction can retard the rate of progression of renal insufficiency, and indeed investigators have observed a rise in 1/Screat after protein restriction suggesting an

amelioration of renal function. However, a reduction of dietary meat intake will lead to a gradual decrease of serum creatinine and a parallel reduction in urinary creatinine excretion. Based on these data, one would expect that reduction of meat intake per se causes an increased 1/Screat, independent of changes in renal function. Indeed, it has been demonstrated that in such cases 1/Screat suggested an improvement of renal function, whereas the measured creatinine clearance actually decreased. Furthermore, we could demonstrate that dietary protein intake influences creatinine secretion. When we prescribed a high protein diet for four weeks to patients with renal failure, we observed that creatinine secretion increased. More recently we have studied the influence of a meat meal on the tubular secretion of creatinine in healthy subjects [4]. Our results indicated that the meat meal almost doubled creatinine secretion. Thus, in clinical studies creatinine cannot not be used as a precise marker of GFR.

Prednisone-induced alterations in proteinuria

Glucocorticoid treatment is regularly used in patients with a nephrotic syndrome. While treating patients with a nephrotic syndrome with corticosteroids in an alternate-day regime, we observed a typical fluctuating pattern of proteinuria, protein excretion on prednisone days exceeding that on non-prednisone days [2]. The percentual change of proteinuria correlated with baseline endogenous creatinine clearance. In view of evidence in the literature, the alterations in proteinuria during alternate day prednisone therapy were initially attributed to a prednisone-induced increase of protein excretion. To unravel the mechanisms of this effect we have studied patients with either membranous glomerulonephritis or minimal change disease and a nephrotic syndrome more closely. The acute proteinuric effects of prednisolone were studied in nine patients. After intravenous administration of 125-150 mg of prednisolone, urinary protein excretion rose in all patients, percentual increases ranging from 21% to 178% [median 95%]. Glomerular

filtration rate and effective renal plasma flow did not change significantly. The percentual increases of urinary excretion of albumin, IgG, and transferrin were in the same range. Urinary excretion of β2-microglobulin did not change significantly, however. These observations suggest that the increase of urinary protein excretion after administration of prednisolone cannot be explained by changes in renal hemodynamics or tubular protein reabsorption, and might be the result of a change in glomerular permselectivity characteristics.

Retrospective analysis of our data suggested that the differences in protein excretion on prednisone and non-prednisone days could also at least partly be the result of a decreased proteinuria on non-prednisone days. The magnitude and the possible mechanisms of the decreased protein excretion on non-prednisone days were studied in more detail in 14 patients with a nephrotic syndrome and membranous glomerulonephritis. In these patients renal hemodynamics and proteinuria were studied at base-line and six days after starting alternate day prednisone treatment on a non-prednisone day. Proteinuria decreased from 7.7 ± 1.9 to 4.9 ± 1.5 mg/min [percentual decrease 45±8%; $p<0.01$]. Glomerular filtration rate and renal plasma flow did not change significantly. However, filtration fraction decreased slightly, but significantly, by 7 ± 2%. Percentual decreases of albumin, IgG, and transferrin excretion were comparable, whereas excretion of β2-microglobulin was less reduced. From these observations we conclude that proteinuria is decreased on a non-prednisone day. This decrease of proteinuria is mediated by a decreased glomerular capillary pressure and a change in glomerular size-selectivity.

On the non-prednisone day endogenous cortisol production was depressed, plasma cortisol decreasing from 0.27 ± 0.04 mmol/l at base-line to 0.10 ± 0.02 mmol/l [$p<0.01$]. Could this change in cortisol levels be related to the alterations in proteinuria? Adrenalectomy reduces proteinuria in rats. Substitution therapy with glucocorticoids but not mineralo-corticoids

restores proteinuria. From these observations it was concluded that glucocorticoids have a permissive effect on proteinuria. The effects of glucocorticoids on proteinuria may be the result of a change in glomerular filtration rate as well as a direct effect on glomerular permeability. The results of our studies suggest that glucocorticoids, in casu prednisone, also interfere with proteinuria in humans. Recent studies have stimulated the interest in the effects of steroids on proteinuria. First, in the remnant kidney model in the rat, a non-immunological model of progressive renal failure, chronic administration of pharmacological doses of methylprednisolone accelerated the development of glomerular sclerosis. Secondly, in human individuals with renal failure a positive correlation was found between the rate of progression of renal insufficiency and the excretion of 17-hydroxysteroids. These data suggest that corticosteroids, even when levels are within the physiological range, may play a role in the deterioration of renal function. If this is the case, even low doses of prednisone, administered daily, could contribute to the progression of renal failure.

The results of our studies could have, therefore, important clinical consequences. Alternate day prednisone therapy may be less detrimental and also new therapeutic approaches using antiglucocorticoid drugs may be worthwile to explore. Clearly, more studies on the effects of prednisone in patients with renal disease are needed to substantiate these speculations.

Clinical and therapeutic aspects.

Membranous glomerulonephritis is the most common glomerular disorder underlying the nephrotic syndrome in adults. The natural history of the disease in untreated patients is quite variable. A rough estimate learns that half of the patients will reach remission of proteinuria, whereas the other half will develop renal insufficiency. Up to this time no definitive answer can be given to the question how to treat these patients. Two controlled trials using prednisone monotherapy have

yielded equivocal results. Therefore we analysed the course of the disease in 34 patients, who had been treated with 125 to 150 mg of prednisone on alternate days. Female patients [n=6] had a good prognosis, none of them progressing to renal failure. However, more than half of the men developed renal insufficiency, and only three reached a complete remission of proteinuria within one year after starting the treatment. It is not justified to draw firm conclusions from such an uncontrolled study. However, it is evident that the majority of patients will not benefit from steroid treatment. Recently the results of a controlled trial have been reported, demonstrating beneficial effects of combined treatment with chlorambucil and prednisone in patients with membranous glomerulonephritis and well preserved renal function. Nearly three quarter of patients reached a complete or partial remission of proteinuria, and only a few developed renal insufficiency. It is evident that these results are superior to those obtained by treatment with prednisone alone. Should we then offer all our patients this treatment regimen? One must be careful to jump to conclusions in this difficult field. First, the results have to be confirmed in other controlled trials. Secondly, membranous glomerulonephritis is a disease with a rather indolent course, and over 50% of the patients will spontaneously improve. If we treat all patients, the latter group will unnecessarily be exposed to the short-term adverse effects and the potential long-term hazards of chlorambucil treatment. Ideally, such aggressive treatment regimens should be restricted to patients with a high likelihood of developing renal insufficiency. As mentioned above, such patients can be identified within two to three years after presentation. Can the start of treatment be delayed safely until renal function starts to deteriorate? Recent uncontrolled studies have furnished important information on this subject. Patients with membranous glomerulonephritis and deteriorating renal function are not invariably resistant to treatment. In fact, in 50 to 70% of patients renal function improved or stabilised after treatment with regimes including cyclophosphamide, azathioprine, chlorambucil, or

prednisone. We have used combined treatment of chlorambucil and prednisone in six patients with renal insufficiency, and noted an improvement or stabilisation of renal function in three of them. Further studies in such patients are needed to confirm the effectiveness of treatment regimens and to select the best possible drug or combination of drugs.

Concluding remarks

Our experience with prednisone monotherapy in patients with membranous glomerulonephritis is somewhat disappointing. Although serious side effects rarely occur, the benefits of treatment are doubtful, so that we cannot longer recommend to treat all patients with idiopathic membranous glomerulonephritis with an eight week course of prednisone. Up to this moment the question remains open, if addition of pulses methylprednisolone could enhance the therapeutic efficacy of prednisone monotherapy. Newer treatment regimes including combined treatment with chlorambucil and prednisone are clearly more effective, even in patients with established renal failure. We feel that it is important to restrict such treatment regimes to patients with a high likelihood of developing renal insufficiency. Patients without [a high risk for] renal insufficiency should not be treated with such aggressive drugs. It seems worthwile to explore prospectively the effectiveness of pulses methylprednisolone in the latter group of patients.

Although the therapeutic effects of prednisone monotherapy did not meet our expectations, the use of it has drawn our attention to rather unexpected acute effects of prednisone on proteinuria. In our view the decrease of proteinuria on non-prednisone days could have important clinical consequences. Our data fit well with the idea that a decreased production of cortisol could attenuate protein excretion and retard progression of renal insufficiency. The introduction of antiglucocorticoid drugs, which specifically antagonise the effects of glucocorticoids at the receptor level, will make it possible to

study further the effects of cortisol on proteinuria. It will be interesting to see whether these antiglucocorticoid drugs can decrease proteinuria. If this is the case, these drugs might be useful for therapeutic intervention. The fact that proteinuria is intermittently decreased during alternate-day prednisone therapy might indicate that such a regime is preferable to daily prednisone treatment. In this regard it would also be interesting to study renal transplant recipients with proteinuria and chronic vascular rejection. These patients use prednisone on a daily basis. It is conceivable that alternate day treatment might retard the progression of renal failure in these patients.

The relevance of our finding of an acute proteinuric effect of prednisone is less clear. The increased proteinuria may simply reflect the damaging potential of prednisone on the glomerular filter. However, in recent studies in patients with proliferative glomerulonephritis we could not detect such an increase of protein excretion after administration of prednisone. This might indicate that the nature of the underlying disease and possibly the nature of the defect in the glomerular basement membrane determines the response to prednisone. These findings call for further studies of the effects of prednisone in patients with renal failure.

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SAMENVATTING

Membraneuze glomerulonefritis is de meest frequente oorzaak van het nefrotisch syndroom bij volwassenen. In 1979 werd een behandelingsschema geïntroduceerd bestaande uit 125 tot 150 mg prednison op alternerende dagen gedurende acht weken. Bij de behandeling van patienten volgens dit schema viel op dat de eiwituitscheiding op prednison-dagen duidelijk hoger was dan op de dagen dat de patienten geen prednison gebruikten. Deze klinische waarneming leidde tot nader onderzoek, waarvan de resultaten in dit proefschrift zijn weergegeven.

Het natuurlijk beloop van de membraneuze glomerulonefritis is wisselend [hoofdstuk II]. Globaal genomen komt de helft van de patienten spontaan in remissie, terwijl de andere helft een nierinsufficientie ontwikkelt. Voor vrouwen is de prognose gunstiger. Het nut van behandeling met prednison alleen is omstreden; nieuwere behandelingsschema's met cyclophosphamide, chloorambucil of azathioprine lijken effectiever en ook werkzaam bij patienten met een reeds bestaande nierinsufficientie.

Wij onderzochten de invloed van de urine flow op de eiwituitscheiding bij patienten met evidente proteïnurie [hoofdstuk III]. De eiwituitscheiding is bij een diurese van meer dan 1,5 ml/min [=2,2 L/24 uur] constant en onafhankelijk van de urineproductie. Bij lagere diurese neemt de eiwituitscheiding af, mogelijk door een toegenomen tubulaire reabsorptie. Bij onderzoek naar veranderingen in de eiwituitscheiding moet dan ook gestreefd worden naar een diurese van meer dan 1,5 tot 2,0 ml/min.

De doorlaatbaarheid van de glomerulaire basale membraan [GBM] wordt ten dele bepaald door de negatieve lading ervan. Een afname van de negatieve lading kan leiden tot fors eiwitverlies, met name door toegenomen lekkage van albumine. Wij onderzochten of de verhouding van de klaring van het neutrale pancreas-amylase [P-amylase] en het negatief geladen speekselklier-amylase [S-amylase] een maat zou kunnen zijn voor de negatieve lading van de GBM [hoofdstuk IV]. Bij proefpersonen

bleek inderdaad de klaring van P-amylase hoger dan de klaring van S-amylase. Bij patienten met een nierziekte en proteinurie bleek de klaring van S-amylase relatief toegenomen, hetgeen in overeenstemming is met het bekende verlies van lading van de GBM bij deze patienten. Het is echter de vraag of de bepaling van de klaringen van P- en S-amylase voldoende gevoelig is om kleine veranderingen in de negatieve lading aan te tonen.

In hoofdstuk V worden verschillende factoren besproken die de waarde van serumcreatinine en de creatinineklaring als maat voor de nierfunctie beperken. Het belangrijkst in dit verband zijn de sterke variabiliteit in de tubulaire secretie en het extrarenale metabolisme van creatinine. Ook het feit dat de voeding, in casu vlees, een belangrijke bron van creatinine is, bemoeilijkt in vele gevallen de interpretatie van nierfunctiegegevens op basis van creatininebepalingen.

Hoofdstuk VI geeft een overzicht van de waarnemingen tijdens de behandeling van patienten met een nefrotisch syndroom met hoge dosis prednison op alternerende dagen. Op prednison-dagen bleek de eiwituitscheiding anderhalf tot twee maal zo groot als op niet-prednison-dagen. De mate van verschil was gecorreleerd met de creatinineklaring. Bij patienten die niet met diuretica behandeld werden leek de eiwituitscheiding, met name op de eerste dag van de behandeling, extra toe te nemen. In een vervolgonderzoek bleek dat intraveneuze toediening van prednisolon binnen vijf uur leidde tot een sterke toename van de proteinurie met gemiddeld 95% [hoofdstuk VII]. De toename van de proteinurie ging niet gepaard met veranderingen in de nierfunctie noch in de uitscheiding van β2-microglobuline. Dit maakt het aannemelijk dat de toename van de proteinurie berust op een verandering in de glomerulaire permeabiliteit.

In hoofdstuk VIII wordt duidelijk aangetoond dat de beschreven wisselingen van de eiwituitscheiding tijdens prednisonbehandeling voor een belangrijk deel berusten op een afname van de proteinurie op niet-prednison-dagen. De afname van de proteinu-

rie gaat gepaard met een verminderde filtratiefractie. Er worden vergelijkbare veranderingen in de uitscheiding van albumine, IgG, en transferrine gevonden. Deze gegevens duiden erop dat de afname van de eiwituitscheiding berust op een verminderde intraglomulaire druk en een verminderde permeabiliteit van de GBM. Een en ander wordt mogelijk veroorzaakt door de waargenomen suppressie van de endogene cortisolproductie op de niet-prednison-dagen. Recente studies bij patienten met een nierinsufficientie wijzen op een verband tussen de endogene glucocorticoidproductie en de snelheid van nierfunctieverlies.

Ondanks behandeling met hoge dosis prednison gedurende acht weken ontwikkelde meer dan de helft van de mannelijke patienten met een membraneuze glomerulonefritis een nierinsufficientie [hoofdstuk IX]. Daarentegen ontwikkelde geen van de zes aldus behandelde vrouwen een nierinsufficientie. Gaat de nierfunctie achteruit, dan gebeurt dit meestal binnen twee tot drie jaar na het begin van de ziekte. Patienten die een nierinsufficientie ontwikkelen worden gekenmerkt door hoge bloeddruk, ernstige, niet-selectieve proteinurie en een verhoogde uitscheiding van β2-microglobuline. Hoewel het een niet-gecontroleerde studie betreft, lijkt de effectiviteit van prednisonbehandeling op deze wijze niet groot. Naar aanleiding van beschreven gunstige effecten van behandeling met de combinatie chloorambucil en prednison bij patienten met een membraneuze glomerulonefritis, behandelden wij een aantal patienten met een dergelijke combinatie van middelen [hoofdstuk X]. Alle patienten hadden een persisterend nefrotisch syndroom en/of een nierinsufficientie ondanks behandeling met prednison alleen. Wij zagen slechts bij één patient een partiele remissie van de proteinurie. Echter, van de zes patienten met tevoren een dalende nierfunctie, verbeterde deze bij twee, en bleef stabiel bij een derde patient. Op dit moment lijkt het daarom het verstandigst deze behandeling te reserveren voor patienten die reeds achteruitgang van de nierfunctie tonen.

De schrijver van een proefschrift is te vergelijken met een sprinter, die een wielerwedstrijd wint en daarmee een sterk ploegenspel doeltreffend afrondt. Velen hebben mij de afgelopen jaren uit de wind gereden en vanaf deze plaats wil ik al mijn ploegmakkers danken. Zonder iemand tekort te doen wil ik met name noemen:
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CURRICULUM VITAE.

Jack Wetzels werd geboren op 19 oktober 1954 te Heerlen. In 1973 behaalde hij het Gymnasium-B diploma aan het St. Bernardinuscollege te Heerlen. In dat zelfde jaar begon hij met de studie Geneeskunde aan de Katholieke Universiteit te Nijmegen [doctoraalexamen 1978, artsexamen 1980]. Van juli 1980 tot september 1981 vervulde hij zijn dienstplicht als arts-assistent Inwendige Geneeskunde in het Militair Hospitaal te Utrecht. Van november 1981 tot mei 1985 en van mei 1987 tot november 1988 was hij in opleiding tot internist in de Kliniek voor Inwendige Ziekten van het St. Radboudziekenhuis te Nijmegen [opleider: Prof. Dr. A. van 't Laar]. Tijdens zijn opleiding en gedurende de twee jaar onderbreking daarvan, verrichtte hij het onderzoek waarvan dit manuscript het resultaat is. Op 1 november 1988 werd hij ingeschreven in het specialistenregister. Sedertdien is hij werkzaam op de afdeling Nierziekten [Hoofd: Prof. dr. R.A.P. Koene] van de Kliniek voor Inwendige Ziekten van het St. Radboudziekenhuis.
Hij is getrouwd met Marjo van Helden, en vader van Jeroen, Tom, Jos, en Rick.

STELLINGEN

I

De bevinding dat suppressie van de endogene cortisolproductie gepaard gaat met een sterke afname van de proteinurie opent therapeutische perspectieven voor patienten met een nefrotisch syndroom.

II

Behandeling van patienten met een membraneuze glomerulonefritis met hoge doses prednison gedurende acht weken is onvoldoende effectief.

III

De achteruitgang van nierfunctie bij patienten met een membraneuze glomerulonefritis is niet onherroepelijk.

IV

Bij patienten die na niertransplantatie recidiverend tekenen van onverklaarde overhydratie of decompensatio cordis vertonen, dient een stenose van de nierarterie uitgesloten te worden.

V

Het beloop van de diabetische nephropathie na niertransplantatie is niet natuurlijk.

VI

Patienten met diabetes mellitus hebben na een geslaagde niertransplantatie waarschijnlijk een verhoogde kans op het ontstaan of het verergeren van perifere circulatie-stoornissen, waarvoor amputaties noodzakelijk zijn.

VII

Intraveneuze toediening van vincristine kan leiden tot spasme van de coronairarterien en tot myocardinfarct.

VIII

De toename van de lithiumklaring na toediening van triamtereen maakt de aanwezigheid van lithiumtransport in de corticale verzamelbuis aannemelijk.

IX

Het is vooralsnog niet afdoende bewezen dat vroegtijdige, strenge eiwitbeperking bij patienten met een nierinsufficientie het voortgaande verlies van nierfunctie vertraagt.

X

Gelet op de belangrijke bijdrage van exogeen creatinine is de benaming ECC [endogenous creatinine clearance] misplaatst.

XI

De term "reserve-capaciteit van de nier" dient afgeschaft te worden.

XII

Toediening van anti-thymocytenglobuline [ATG] vormt een effectieve therapie van afstotingsreacties bij niertransplantatiepatienten die met cyclosporine behandeld worden.

XIII

Een toegenomen oxidatief metabolisme in de tubuluscellen speelt waarschijnlijk een belangrijke rol bij de meestal onafwendbare progressie van chronische nierinsufficientie.

XIV

Ook uit wetenschappelijk oogpunt is het schrijven van een proefschrift niet de meest nuttige vrije-tijdsbesteding.

24 november 1989 J.F.M. Wetzels.